The effect of replacing electrolytic iron, in a multi-micronutrient fortification mix, with sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) on the sensory properties of porridge from maize meal

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DECLARATION

I declare that the dissertation herewith submitted for the degree of MSc Food Science at the University of Pretoria has not been previously submitted by me for a degree at any other university or institution of higher education.

SIGNATURE..

DATE...

DEDICATION

Dedicated to my family.

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ABSTRACT

The effect of replacing electrolytic iron, in a multi-micronutrient fortification mix, with sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) on the sensory properties of porridge from maize meal

by

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In developing countries, iron deficiency (ID) and the related iron deficiency anaemia (IDA) affects about 50 % of children and woman and about 25 % of men. In total, an estimated two billion people is affected worldwide. In developing countries, a typical diet is based on cereals, legumes and vegetables and these contain many iron inhibitors like phytic acid and polyphenols. Iron fortification of maize meal, a staple food in South Africa is regarded as a cost effective, sustainable way to improve iron status in that country. The replacement of electrolytic iron with sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) (a more bioavailable source of iron) in the current multi-micronutrient fortificant premix added to maize meal as an iron fortificant compound has the potential to greatly enhance the efforts to improve the iron status of populations consuming high-phytate cereal-based diets. However, poor consumer acceptance, unacceptable taste and discolouration of iron fortified foods have been frequently listed as causes of unsuccessful iron fortification programmes. This study evaluated the effects of replacing electrolytic iron (35 mg iron/kg maize meal) in a multi-micronutrient fortificant premix with NaFeEDTA (at 15 and 30 mg iron/kg maize meal) added to special maize meal on the colour (L* a* b*) and sensory properties (appearance and flavour) of stiff porridges. The porridges were prepared using three different types of cooking vessels (stainless steel, cast iron and aluminium pots) and was evaluated fresh (within 30 minutes of preparation) and after 24 hour refrigerated storage (3 - 5 ^{o}C). The porridges were subjected to analytical sensory evaluation to test for difference or similarity and a consumer acceptance test (n = 80 consumers).

Electrolytic iron significantly reduced L* and a* colour values in fortified maize meal when compared to maize meal fortified with the multi-micronutrient mix excluding iron. Elemental

iron powders (e.g. electrolytic iron) being black or dark grey powders may have caused slight darkening of cereal flours. The spot iron test showed a uniform distribution of iron in maize meal with NaFeEDTA 15 (fortified with 15 mg iron/kg maize meal), NaFeEDTA 30 (fortified at 30 mg iron/kg maize meal) and electrolytic iron as well commercially fortified maize meals.

Electrolytic iron or NaFeEDTA 15 and 30 maize meals resulted in stiff maize porridge prepared in aluminium, cast iron and stainless steel having lower L* values when compared to porridge prepared from maize meal fortified with the multi-micronutrient premix excluding iron. This could be attributed to interaction between polyphenols (ferulic and p-coumaric acid) found in maize meal and iron ions (Fe^{2+}/Fe^{3+}). This interaction has been suggested to induce structural changes and polymerisation in the polyphenols and thereby influencing their light absorption pattern and thus leading to darkening of maize porridges. Cast iron cookware showed significant differences ($p < 0.05$) between iron treated porridges and control porridge (fortified with premix excluding iron) in both appearance and flavour while stainless steel showed significant differences ($p < 0.05$) between iron treated porridges and control porridge only in flavour and aluminium cookware showed significant differences ($p < 0.05$) between iron treated porridges and control porridge only in appearance. These differences could be attributed to the differences in thermal conductivity and heat transfer of the materials of the different cooking vessels and possible leaching of iron ions (Fe^{3+}/Fe^{2+}) from cast iron and stainless steel cookware and aluminium (Al^{3+}) ions from aluminium cookware at different concentrations and/or rates during the cooking process. Appearance changes are probably due to the interaction between ion irons and polyphenols while flavour changes could be attributed to possible lipid oxidation of linoleic acid, a major polyunsaturated fatty acid found in maize meal. Iron ions (Fe^{3+}/Fe^{2+}) promotes lipid oxidation of linoleic acid leading to the development of hexanal, a major off-flavour compound in iron fortified maize meal porridge.

Findings from this study indicate that a change from fortifying maize meal with electrolytic iron to NaFeEDTA will not lead to changes in appearance and flavour of maize porridge. However, an excess amount of NaFeEDTA (30 mg iron/kg maize meal) might lead to acceptability (taste) problems if porridge is prepared in cast iron cookware. Fortification of maize meal porridge with a more bioavailable source of iron (NaFeEDTA) may reduce the prevalence of iron deficiency in South Africa.

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CHAPTER 1: INTRODUCTION

Iron (Fe) deficiency (ID) and the related iron deficiency anaemia (IDA) is the leading nutritional deficiency in the world, affecting an estimated two billion people, with the highest prevalence in the developing world (WHO, 2001). The most affected populations in the developing countries are women and children. This is mainly because the diets are largely based on cereals, legumes and vegetables that contain many Fe-absorption inhibitors like phytic acid (Cagnasso, Calvino, Lopez, Cellerino, Dyner, Binaghi, Rodriguez, Drago, Gonzalez and Valencia, 2013). IDA has adverse effects on pregnancy outcomes, infant growth, cognitive performance, immune status and work capacity. Iron fortification of staple foods is considered a sustainable approach to combat ID (WHO, 2001).

Maize meal, the main product obtained from white maize (*Zea mays* L) kernels is used to make stiff porridge which is the main staple food in the southern Africa region (FAO, 1995; Onyango, 2014). Fortification of maize meal with electrolytic iron is mandatory by law in South Africa (at a minimum concentration of 35 mg iron/kg for special and super maize meal) (Department of Health South Africa, 2003). Technically, iron is the most difficult mineral to add to foods and ensure adequate absorption (Hurrell, 2002a). The element is highly reactive and very unstable (Hurrell, 1999). The main problem is that water soluble iron compounds such as ferrous sulfate, which are the most bioavailable, often lead to the development of unacceptable colour, aroma and flavour changes in the food vehicle (Hurrell, 1997). Iron fortification has been associated with metallic aftertaste (Moretti, Lee, Zimmermann, Nuessli, and Hurrell, 2005) and also with a metallic retronasal smell in addition to astringency (Cagnasso *et al.*, 2013). Baby cereals turned green or gray when ferrous sulfate was added (Hurrell, 1989); iron fortified maize porridge developed an undesirable colour (Viteri, Xunian, Tolomei, and Martin, 1995b).

Concerns, based on anecdotal evidence, were raised that stiff maize porridge from flour fortified with iron tends to develop undesirable colour and flavour changes when stored overnight. It was hypothesized that such colour changes might be linked to the iron fortificant added to the maize meal. Hurrell, (1997) suggested that polyphenols may be involved in the off-colour development of iron fortified foods. A study done in model solutions and in foods rich in polyphenols confirmed this suggestion by showing that ortho-hydroxy groups as found in gallic acid (e.g. in chocolate), catechin (found in green tea), chlorogenic acid (in coffee) cause off-colour developments with iron (Mellican, Li, Mehansho and Nielsen, 2003). The major polyphenols found in white maize meal are ferulic and p-coumaric acid (Pozo-Insfran, Brenes, Saldivar and Talcott, 2006) and these have the ability to undergo oxidation/reduction reactions in the presence of iron ions (Mellican *et al.*, 2003) and this could possibly lead to formation of off-colours in maize porridge. Maize also contains polyunsaturated fatty acids (PUFAs) in the form of linoleic acid (Bovell-Benjamin, Allen, Frankel and Guinard, 1999). Iron ions (Fe^{2+}/Fe^{3+}) in contact with the PUFAs have the capacity to promote destructive free-radical reactions, which can result in the development of off-flavours (Rekhif, Sher, Vadehra and Wedral, 2002).

Because of its low reactivity with food matrices and its low price, electrolytic iron is often chosen for fortification of cereal flours (Hurrell, Bothwell, Cook, Dary, Davidsson, Fairweather-Tait, Hallberg, Lynch, Rosado, Walter and Whittaker, 2002). However, although electrolytic iron is inexpensive, its bioavailability is questionable because it can bind to phytates in cereals. In a study by Andango, Osendarp, Aya, West, Mwaniki, De Wolf, Kraaijenhagen, Kok, and Verhoef, (2007), conducted in Kenya, consumption of maize porridge fortified with electrolytic iron did not improve the iron status of children, aged 3 - 8 years. Maize contains high levels of phytic acid (myo-inositol 6-phosphate), this compound binds strongly to iron and thereby reduce its absorption and bioavailability (Hurrell, Reddy, Burri, and Cook, 2000). In such cases, sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) might be a better fortificant than electrolytic iron for supplementation of iron, because EDTA chelates iron, and might prevent it from binding to phytates. Isotope studies suggest that iron absorption from NaFeEDTA might be two to three times higher than from electrolytic iron (Hurrell, 2002b).

The aim of this study was to investigate the effect of substituting electrolytic iron with NaFeEDTA as part of a prescribed multi-micronutrient fortificant premix on the sensory properties of stiff maize porridge.

The dissertation includes a literature review (chapter 2) leading to the formulation of hypotheses (chapter 3) that will be tested in two experimental phases (chapter 4). A general discussion to critically review the experimental design and methodologies as well as results is presented in chapter 5. Chapter 6 presents conclusions from the research and recommendations for future studies.

CHAPTER 2: LITERATURE REVIEW

In this review, the focus is on the importance of iron fortification of special maize meal - a staple food in South Africa. Iron fortification of maize meal could potentially help to reduce the burden of iron deficiency (ID) and iron deficiency anaemia (IDA) in maize meal eating populations. It highlights the potential impact of replacing electrolytic iron (current legislated iron compound) with NaFeEDTA in the fight against ID and the related IDA in the region. It also highlights the technical challenges that are encountered during iron fortification of food vehicles. The bioavailability of the commonly used iron fortificant compounds is also discussed. The World Health Organisation (WHO) recommends the use of NaFeEDTA (high bioavailable form of iron) in the developing world like Africa (WHO, 2001). The iron fortificant compound (NaFeEDTA) appears to be highly suitable for the fortification of cereal based foods and these form part of the basic diet in most regions of the developing world. The use of NaFeEDTA as an iron fortificant compound has potential to greatly enhance the efforts to improve the iron status of populations consuming high-phytate cereal-based diets. An overview of potential benefits of NaFeEDTA and its potential effects on the sensory properties of cereal flours and cereal products is given. The dry maize meal making process is explained.

2.1 Iron function, iron deficiency and iron bioavailability

2.1.1 The function of iron in the human body

Iron is considered a micronutrient because the human body only needs a very small amount (Guthrie and Picciano, 1995). South Africa's recommended daily intake for iron is 10 mg for children and 18 mg for adults, and it is dependent on age and sex (Department of Health South Africa, 2003). Although the body contains a relatively small amount of iron, iron is present in every cell of the body and is critical for normal function. The main function of iron is the transport and storage of oxygen (Beard, Dawson and Pinero, 1996). In the blood, iron is bound to haemoglobin, which carries oxygen. In the muscles, iron is part of myoglobin, which stores oxygen. The storage of oxygen in myoglobin allows for more efficient use of muscles (Guthrie and Picciano, 1995). In addition, iron as a cofactor is involved in converting the energy stored in foods into useful energy from adenosinetriphosphate (Guthrie and

Picciano, 1995). Iron is an ideal compound to act as a cofactor in many other biochemical reactions because of its flexible oxidation state, oxidation/reduction potential and electron spin state (Beard *et al.*, 1996).

2.1.2 Iron deficiency and iron deficiency anaemia

Iron (Fe) deficiency (ID) and the related iron deficiency anaemia (IDA) is the leading nutritional deficiency in the world (WHO, 2001). The WHO estimates that about 2 billion individuals or about 40 % of the world's population suffer from anaemia (WHO, 2001). Women and children, owing to their increased nutritional requirements for reproduction and growth respectively, are more prone to IDA. IDA is a major cause of low birth weight and maternal mortality and has been recognised as an important cause of cognitive deficit in infants and young children. IDA has a profound effect on productivity and, therefore has economic implications for countries where it is a significant health problem (Darnton-Hill, Mora, Weinstein, Wilbur and Nalubola, 1999). One of the main reasons for the increased interest is the realisation that ID contributes substantially to the global burden of disease. Another reason for the increased attention to the problem of ID is that, contrary to previous thinking, it is not uniquely the concern of poor countries. While ID is certainly more frequent and severe among disadvantaged populations, it does represent a public health problem in some industrialized countries (Guilbert, 2003).

Approximately 50 % of the populations in the less developed countries of South Asia and Africa suffer from anaemia compared with about 25 % in Latin America and approximately 10 % in the industrialised countries of Europe (Hurrell, 1997). Compiling data about the global prevalence of anaemia and ID can be challenging due to the fact that only a few countries collect data on their anaemia prevalence. However, non-representative estimates can be generated from isolated reports and hospital records (Allen and Casterline-Sabel, 2001).

2.1.3 Dietary sources of iron and bioavailability of iron

There are two types of dietary sources of iron: haem and non-haem iron. Haem iron comes from animal sources such as beef, chicken, and fish and it occurs in haemoglobin and myoglobin. It usually constitutes only 5 - 15 % of the dietary intake of iron, but is relatively efficiently absorbed. Iron absorption from haem iron ranges between 15 - 45 % depending on iron status (Garrow and James, 2000; Andrews and Schmidt, 2007). Non-haem iron is found in plant-derived foods, such as grains, vegetables and fruits. The efficiency of its absorption is low and markedly influenced by the dietary constituents that can either enhance or inhibit its absorption (Han, Failla, Hill, Morris and Smith, 1995). The iron from haem sources is absorbed by the body much better than iron from non-haem sources (Baynes and Bothwell, 1990).

Bioavailability is a function of food digestibility, nutrient availability and the ability to use the nutrient for metabolic functions. The extent, to which iron in the diet can improve iron status, therefore will reflect its bioavailability. Whilst food shortage can contribute to ID, it is generally recognised that the poor bioavailability of dietary iron is the key problem causing IDA in many regions of the world. The two main determinants of low bioavailability are the form of dietary iron and the presence of substances such as phytates and polyphenols that bind iron and reduce its absorption. In plant based diets, which predominate in the developing countries, non-haem iron forms the bulk of the ingested iron. These diets however are mostly based on cereals and legumes that contain substantial amounts of iron absorption inhibitors. Poor bioavailability of non-haem iron is one of the major contributing factors to the problem of ID and its improvement is the focus of programmes to improve the iron status of deficient populations (Cagnasso *et al.*, 2013; Lee and Clydesdale, 1978).

2.1.4 Iron absorption and metabolism

Many factors influence absorption of iron, including the individual's need for iron and composition of diet. Important factors affecting the availability of iron include the valence, solubility and degree of chelation or complex formation of the iron (Andrews and Schmidt, 2007; Lee and Clydesdale, 1978). It has been shown that the ferrous valence is much more available than the ferric valence. It appears that before iron can be absorbed in the gut, it must be in solution. Chelation may enhance iron absorption by maintaining the iron in solution under conditions where otherwise it would be insoluble (Lee and Clydesdale, 1979).

Virtually all plant food-derived iron is in the ferric (Fe^{3+}) form, which must be reduced to the ferrous (Fe^{2+}) form before it can be absorbed by enterocytes, a type of intestinal cells. The gastrointestinal environment is an important factor in determining how much iron will eventually be absorbed. In solutions with a pH greater than 3, ferric iron forms insoluble iron hydroxides and is precipitated from solution, hence is unavailable for absorption. This may be prevented in two ways: chelating of ferric iron, at low pH in the stomach by dietary and intestinal derived substances which keep iron in solution when it enters the less acidic duodenum (Conrad and Umbreit, 2002), or by reduction of ferric iron to ferrous iron. In normal circumstances the pH of the stomach is low therefore favourable for the ferric iron chelation and solubilisation. Factors that reduce the acidity of the stomach can therefore reduce the bioavailability of non-haem iron. Some ferric iron is reduced by dietary constituents and intestinal secretions to ferrous iron which is soluble in neutral pH. However, in the absence of either continuous reduction or chelation to prohibit exposure of the iron to oxygen, ferrous iron is rapidly oxidised to ferric iron (Anderson, Frazer, Mckie, Vulpe and Smith, 2005). The stomach and to some extent the duodenal environment may also favour inhibition of iron absorption: other dietary constituents such as phytates, which are already mentioned, are abundant in cereal and grain based diets; polyphenols, calcium and some proteins, may form complexes with iron which render it unavailable for absorption. To improve the bioavailability of non-haem iron, it must be reduced to Fe^{2+} or a sufficient amount of ligands must be present in order to maintain Fe^{3+} in the soluble phase (Conrad and Umbreit, 2002).

Whilst increasing iron intake in deficient individuals improves iron status, there are concerns that it may also result in iron overload. Because no iron-excretory mechanism exists, the body must tightly regulate the amount of iron that eventually enters the circulation, because excess iron resulting in iron overload is toxic (Andrews and Schmidt, 2007).

2.2 Factors in the diet that affect iron absorption

2.1.1 Phytic acid

Phytic acid (myo-inositol hexa-phosphate), is a major inhibitor of non-haem iron absorption (Hurrell *et al.*, 2002). It constitutes 1 - 2 % of many cereals, legumes and seeds, and has the function of a phosphorous store for the germinating plant. Phytate inhibition of iron absorption is dose dependent (Hallberg, Brune and Rossander, 1989), and is assumed to be due to the complexation and precipitation of ferric-phytate salts in the small intestine with increasing pH (Conrad and Umbreit, 2000). In the duodenum, phytate can form complexes with cations and proteins (Cheryan, 1980), but as yet no evidence has been found on a differentiated effect of phytic acid with different types of proteins (Reddy, Hurrell, Juillerat and Cook, 1996). In addition to phytic acid chelation, dietary fibre also contributes to low iron bioavailability in maize. However the combination of organic acids, such as ascorbic acids, with high-fibre whole grain maize meal showed an increased iron bioavailability in humans by limiting the binding affinity of iron for intrinsic kernel fibres (Reinhold, Garcia and Garzon, 1981).

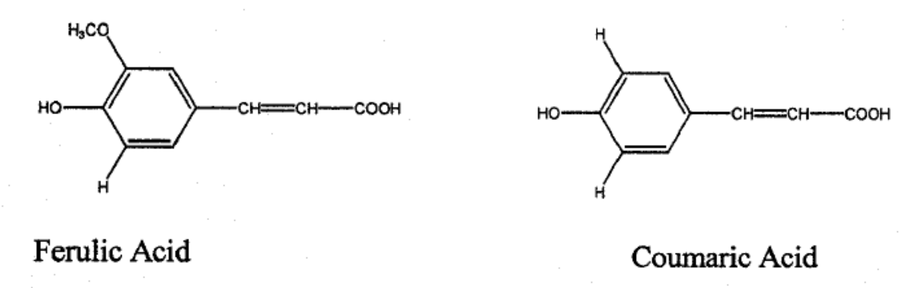
Extraction rate at milling strongly decreases both phytic acid and iron content in cereals. Iron absorption from different cereal grains with or without food processing can be predicted by the phytate content (Cook, Reddy, Burri, Juillerat and Hurrell, 1997; Hurrell *et al.,* 2002). Studies in single meals indicate that phytic acid must be almost entirely removed from meals to eliminate its inhibiting effect on iron absorption. It has been suggested that the phytic acid to iron molar ratio must be reduced to < 0.7:1 to achieve at least a two-fold increase in iron absorption (Hurrell, 2002b). Phytic acid can be degraded by native or fungal phytase. Phytases are active at slightly acid pH (5.1) and are inactive in dry cereals (Oatway, Vasanthan and Helm, 2001).

2.2.2 Polyphenols

Polyphenols can be classified as phenolic acids, flavonoids and complex polyphenols. All classes have been shown to inhibit iron absorption in a dose dependent fashion, depending on their structure, whereas the presence and number of orthodihydroxy-groups has been reported to be critical for inhibition (Bothwell, Baynes, MacFarlane and MacPhail, 1989; Brune, Rossander and Hallberg, 1989). Several studies have reported the antioxidant and anticarcinogenic effects of white maize *(Zea mays* L) polyphenols such as ferulic and p-coumaric acid along with their respective derivatives (Pozo-Insfran, *et al.,* 2006). Figure 2.1 shows the structures of ferulic and p-coumaric acid (Mellican *et al.,* 2003). Many of the polyphenol compounds in maize are covalently bound to cell wall polysaccharides and function in the kernel as cross-linkers to strengthen the grain cell wall (Arendt and Zannini, 2013). Maize contains around about 31 mg/100g polyphenols on dry matter basis (Mellican *et al.,* 2003).

Tannins of black tea, for example, are the most potent iron absorption inhibitors (Brune *et al.,* 1989). Phenolic compounds in red wine inhibit iron absorption compared to water and white wine (Cook, Reddy and Hurrell, 1995), and polyphenols are the absorption inhibiting factor in coffee (Morck, Lynch and Cook, 1983), black tea (Disler, Lynch, Charlton, Torrance, Bothwell, Walker and Mayet, 1975), herbal tea (Hurrell, Reddy and Cook, 1999) and cocoa (Gillooly, Bothwell, Charlton, Torrance, Bezwoda, MacPhail, Derman, Novelli, Morrall and Mayet, 1984; Hurrell *et al.,* 1999). It has been shown that in beverages containing 20 - 50 mg polyphenols per serving, iron absorption was reduced by 50 - 70 % (Hurrell *et al.,* 1999).

Polyphenols-containing vegetables have been shown to inhibit iron absorption (Gillooly, Bothwell, Torrance, MacPhail, Derman, Bezwoda, Mills, Charlton and Mayet, 1983), and the authors report a strong correlation (R = 0.86, P < 0.001) between the total polyphenols content and the iron absorption form different vegetables. Iron absorption from sorghum, an important food crop in Central America, Africa and South Asia, is dependent on its polyphenols content, and it has been shown that low polyphenols-containing varieties are better sources of bioavailable iron (Gillooly *et al.*, 1984).



Ferulic Acid

Coumaric Acid

Figure 2.1: Structures of monomeric polyphenols commonly found in white maize (*Zea mays* L)

2.3 Fortification as a strategy to counteract iron deficiency

There is a consensus that food fortification can be an effective long term approach to increase the iron status of a population. Ideally, fortification reaches all segments of the population and does not require the constant cooperation from the individual or drastic changes in food habits (Mannar and Sankar, 2004; WHO, 2001). Four types of food fortification are recognized by the WHO: mass or universal fortification refers to foods consumed by the entire population, it is regulated by the government and is encouraged in countries where several population segments are at risk of deficiency; open market fortification is practiced mainly in developed countries (e.g. breakfast cereals and functional foods), it is done by the private sector with the aim of increasing the public appeal and the added value of food products; targeted fortification is directed specifically to high risk groups (e.g. infants and pregnant women), whereas with household fortification, the nutrients are added immediately before consumption (Lynch, 2005).

Several factors are critical for a successful fortification programme. For effective fortification, it is important that the strategy identifies a combination of fortificant and vehicle that is acceptable to the target population, and a form of iron that is bioavailable (MacPhail and Bothwell, 1989). The fortificant food must be commonly consumed in constant patterns with low risk of over-consumption; the food vehicle should be centrally produced and fortification must be possible at relatively low cost (FAO, 1996). A fortification program must be adapted to the local food consumption patterns, to the prevalence of deficiency, and, in the case of iron, to the bioavailability from the local diet (Lofti, Mannar, Merx, and Naber-van den Heuvel, 1996). Even if very cost effective (Darnton-Hill, 1998) fortification will marginally increase prices (Underwood and Smitasiri, 1999). Country level experiences for long term success with fortification show that political will, involvement of the private sector at early stages, willingness to enforce quality standards and consumer awareness are important in the implementation of a fortification program (Darnton-Hill, 1998; Underwood and Smitasiri, 1999). Ideally, food fortification should be embedded in an overall strategy to promote nutritional health that includes diet diversification, fortification and supplementation (Mannar and Sankar, 2004).

2.4 Iron fortification compounds

Most commonly recommended iron fortificants include elemental iron, ferrous sulfate, ferrous fumarate and more recently NaFeEDTA (Hurrell, 2002a). Some examples of some of the most frequently used iron compounds for fortification and their characteristics are shown in (Table 2.1) (Kiskini, Kapsokefalou, Yanniotis and Mandala, 2010; Bothwell and MacPhail, 2004). In order to enter the common non-haem iron pool, iron has to be soluble in the gastric juice. The solubility of iron fortification compounds is a primary determinant of their bioavailability (Swain, Newman, and Hunt, 2003). For this reason iron fortification compounds are often classified according to their solubility in water (Hurrell, 1999). A further critical measure used to judge an iron fortification compound is the relative bioavailability (RBV), which is defined as the relative absorption of a certain iron compound in comparison to the same dose of ferrous sulfate, which per definition has a relative bioavailability of 100 % (Hurrell, 1999).

Ferrous sulfate is the cheapest iron salt. To avoid unacceptable colour changes and oxidative reactions it can only be used in cereal flours that are used within one month from production and in low moisture foods as noodles and pasta (Hurrell *et al.*, 2002). Ferrous sulfate and ferrous fumarate are highly bioavailable in the absence of iron absorption inhibitors (International Nutritional Anaemia Consultative Group, 1993). Ferrous fumarate is not completely free from sensory problems, but interacts to a lesser extent with the food matrix than ferrous sulfate. Ferrous fumarate is widely used as iron fortificant in infant cereals in Europe and in chocolate drink powders (Hurrell *et al.*, 1999). It has also been used in precooked corn flour and wheat flour fortification in Venezuela (Garcia-Casal and Layrisse, 2002). Although highly bioavailable, the use of ferrous sulfate and ferrous fumarate is limited to low-phytate diets because they are all susceptible to binding by iron absorption inhibitors, hence their bioavailability is substantially reduced in high-phytate diets (Fidler, Davidsson, Zeder, Walczyk and Hurrel, 2003).

Table 2.1: Most commonly used iron fortificants and their solubility and bioavailability

		Relative bioavailability*	
Iron fortificant	Water solubility	in rates	in humans
Ferrous sulfate	Water soluble	100	100
Ferrous gluconate	Freely water soluble	97	89
Sodium iron EDTA	Slowly soluble in water	150-300	150-300
Ferrous fumarate	Poorly soluble in water	95	100
Ferrous saccharate	Soluble in dilute acid	92	74
Ferric pyrophosphate	Water soluble	45	21-74
Electrolytic iron	Poorly soluble in dilute acid	8-79	5-90

*Relative to ferrous sulfate = 100, for the same level of total iron.

(Kiskini *et al.*, 2010; Bothwell and MacPhail, 2004)

The main determinants of solubility of elemental iron powders in the gastric juice are particle size distribution, surface area, purity and solubility in acid (Hurrell, 2002a). Elemental iron powders are manufactured by five different processes: H-reduction, CO-reduction, atomisation, electrolytic and carbonyl processes (Hurrell, 2002a). Every process results in products with distinct physical characteristics (Hurrell *et al.*, 2002). Elemental iron powders were initially the iron fortificant of choice because of their stability in food vehicles, their low cost and because they cause few sensory problems. Although this form of iron increases the overall iron content of the diet, the absorption of such iron, is highly dependent on meal composition. Of the elemental iron powders, electrolytic iron has reasonable efficiency in improving iron status when used in some food vehicles and its stability and sensory properties favour its continued use. The compound is water insoluble and poorly soluble in dilute acid (Table 2.1). Poorly soluble iron fortification compounds have lower bioavailability compared to ferrous sulfate, but are nonetheless widely used in food enrichment and fortification due to the negligible sensory problems they cause in food vehicles (Hurrell, 2002a). Electrolytic iron is susceptible to binding by iron inhibitors; hence its bioavailability is substantially reduced in high-phytate diets (Fidler *et al.,* 2003). Currently, electrolytic iron is the preferred iron compound for low-phytate flour and is the form of iron legislated for fortification of maize and wheat flours in South Africa while the use of ferrous fumarate is optional (Department of Health South Africa, 2003).

Electrolytic iron was recommended based on the evidence showing the compound to have a bioavailability of 75 % in humans (Hurrell *et al.,* 2002). Swain *et al.,* (2003) reported that electrolytic iron had 54 % of the bioavailability of ferrous sulfate based on rat studies. More research has shown electrolytic iron to be effective in improving iron-status in humans. Hoppe, Hulthen and Hallberg (2005) found that electrolytic iron had 65 % absorption in Swedish subjects consuming fortified rolls, compared with ferrous sulfate. A randomized, controlled efficacy trial showed that electrolytic iron has a relative efficacy to ferrous sulfate of 77 % in humans. This study was performed in Thai-women which were supplied a mid day cookie fortified with 12 mg iron as either electrolytic iron, H-reduced iron, ferrous sulfate or a unfortified control (Zimmermann, Chaouki and Hurrell, 2005). However, the bioavailability of electrolytic iron reported in the literature varies greatly. One study conducted in Kenya, found that maize porridge fortified with electrolytic iron did not decrease the prevalence of IDA in children and it did not improve any of the iron status indicators evaluated (Andango *et al.,* 2007).

NaFeEDTA has been reviewed and approved by the JECFA (Joint FAO/WHO expert committee of food additives) for government supervised fortification programmes. In high-phytate diets, absorption of iron from NaFeEDTA is 2 - 3 times the absorption from ferrous sulfate; in the absence of phytates, its absorption is comparable to that of ferrous sulfate (Table 2.1) (Hurrell *et al.,* 2000). Interestingly, NaFeEDTA compared to ferrous sulfate does only slightly improve iron absorption in foods rich in polyphenols (Hurrell *et al.,* 2000), probably because polyphenols have greater affinity to non-haem iron than EDTA (Bothwell and MacPhail, 2004). NaFeEDTA has been recommended for use in soy and fish sauces. It is also proposed for high phytate flours and other condiments (Hurrell, Lynch, Bothwell, Cori, Glahn, Hertrampf, Kratky, Miller, Rodenstein, Streekstra, Teucher, Turner, Yeung and Zimermann, 2004), because it has the potential to greatly enhance efforts to improve the iron status of populations consuming high-phytate cereal based diets (Hurrell *et al.,* 2004).

There are a number of technical issues related to the use of NaFeEDTA as a fortificant. They relate, on the one hand, to the stability of the complex in a number of vehicles during processing, storage and cooking and, on the other, consumer acceptance of the fortified food in relation to its physical, sensory, and chemical properties. NaFeEDTA, which is pale yellow in colour, causes fewer sensory problems than other water soluble iron compounds. NaFeEDTA is stable at cooking temperatures of 100 ^{o}C but processing fortified food at significantly higher temperatures may cause problems (Bothwell and MacPhail, 2004).

EDTA has the highest affinity for iron in the acid environment of the stomach. It can however be exchanged with other metals in the duodenum. On molar basis, copper and zinc could be affected by the addition of EDTA. Other minerals such as calcium and magnesium would not be affected by the low amounts of EDTA when given at quantities to supply the recommended dietary allowance (RDA) for iron. There are indications that EDTA increases the absorption from copper and zinc in meals rich in phytic acid (Bothwell and MacPhail, 2004). Concern has been expressed on the possible effect of EDTA on the absorption of toxic metals. Although not many studies have been performed on the subject, in a human study with isotopically labeled lead (Pb), simultaneous ingestion of EDTA markedly decreased lead retention (Flanagan, Chamberrlain and Valberg, 1982). This would therefore indicate that EDTA does not increase the absorption of lead. An additional argument leading in this direction is that a small amount of food EDTA is absorbed in the gut, reaches the blood stream and is successively excreted in the urine (Bothwell and MacPhail, 2004). EDTA is normally used for complexation therapy in cases of acute heavy metal poisoning. This would therefore suggest that food EDTA might lower blood lead levels. This question merits further attention because of the widespread coexistence of elevated blood lead levels and ID in urban environments (Kwong, Friello and Semba, 2004).

Several successful iron fortification trials with NaFeEDTA have been performed, including one in an Indian community in South Africa receiving fortified curry powder (Ballot, MacPhail, Bothwell, Gillooly and Mayet, 1989). Fish sauce and soy sauce also can be fortified with NaFeEDTA. A randomized controlled trial has shown that fish sauce fortified to provide 10 mg iron/day significantly increased haemoglobin and significantly decreased prevalence of anaemia in garment factory workers in Thailand (Van Thuy, Berger, Davidsson, Khan, Lam, Cook, Hurrell and Khoi, 2003). These findings were confirmed in a completed effectiveness trial (Van Thuy, Berger, Nakanishi, Khan, Lynch and Dixon, 2005). NaFeEDTA has also been reported to be a suitable fortificant for sugar (Viteri, Alvarez and

Batres, 1995a), although the successful controlled study was difficult to interpret due to logistic and acceptability problems (Bothwell and MacPhail, 2004). Van den Wijngaart and Codling (2013) reported that flour fortified with NaFeEDTA at 40 mg iron/kg produced wet noodles with a slightly darker colour when compared to wet noodles produced from unfortified flour; flour fortified with NaFeEDTA at 20 mg iron/kg produced wet noodles with a similar colour to wet noodles produced from unfortified flour. Buns prepared from flour fortified with NaFeEDTA at 40 mg iron/kg were slightly darker in colour when compared to buns prepared from unfortified flour and there were no significant differences in colour of pittu (steamed cylinders of ground rice layered with coconut) made from flour fortified with NaFeEDTA at 20 mg iron/kg and control flour.

The disadvantages of NaFeEDTA are its high cost, and the lack of systematic information on its sensory proprieties. When sugar fortified with NaFeEDTA was added to coffee and tea, sensory changes were visible (Viteri *et al.*, 1995a). Additionally, milk and salt cannot be fortified with NaFeEDTA due to adverse sensory changes (Bothwell and MacPhail, 2004). In cereal products no rancidity in flour could be detected after 6 months storage at 37 ^{o}C (Hurrell, 1997), but there are reports of colour development in cereal based foods (Viteri *et al.*, 1995a). Additional research is however needed on the stability of NaFeEDTA in a wider range of products and during processing and cooking, especially in cereal based products (Hurrell *et al.*, 2004). In condiments such as curry powder, soy and fish sauce NaFeEDTA appears nevertheless to be well tolerated (Bothwell and MacPhail, 2004).

2.5 Safety of NaFeEDTA

Central to the use of any food fortificant is the issue of safety. Safety aspects of NaFeEDTA concern both its iron and its EDTA component. Iron from NaFeEDTA has been shown to have a similar level of toxicity to that of ferrous sulfate (Whittaker, Ali, Imam and Dunkel, 2002), and concentrations of up to 140 mg iron/kg from either ferrous sulfate or NaFeEDTA were not found to result in excessive loading in rats (Appel, Kuper and Woutersen, 2001). Early studies to assess the toxicity of EDTA have been reviewed comprehensively (Heimbach, Rieth, Mohamedshah, Slesinski, Samuel-Fernando, Sheehan, Dickmann and Borzelleca, 2000). In animal studies, levels higher than used for fortification with NaFeEDTA did not affect growth, reproductive performance, or allergenicity; neither was there any evidence of genotoxicity (INACG, 1993). Although evidence of the potential usefulness of NaFeEDTA was available in the early 1970s, concerns about its safety stalled its use in fortification programmes. In the gastrointestinal tract, NaFeEDTA splits into iron and EDTA. Only a small fraction (< 5 %) of EDTA is absorbed, this absorbed EDTA is rapidly and completely excreted in the urine and does not accumulate in the body. Iron and EDTA are absorbed by separate and independent mechanisms when NaFeEDTA is added to a meal (INACG, 1993). Thus risk assessment for excess intake of NaFeEDTA should separately address the risk for excess intake of iron and EDTA. Although there is growing evidence that NaFeEDTA may not influence the metabolism of other nutrient elements (Davidsson, Almgren and Hurrell, 1998), evidence of its potential effect in young children is lacking.

2.6 Sensory aspects of iron fortification

Foods can be enriched or fortified with nutrients. Foods that are enriched have nutrients added back to them so that the finished product contains the same amount of nutrients as the food did prior to processing. Fortified foods have nutrients added to them to provide additional nutrients to the consumers (Mellican *et al.,* 2003). Due to the vast array of iron compounds and of potential fortification vehicles, a systematic methodology in the identification of the most promising approaches was proposed (Bovell-Benjamin and Guinard, 2003). Among sensory attributes, colour and taste is the most important reason for selecting a food product, and sensory proprieties are generally critical in determining consumers' acceptance of the food. The major technical challenge in iron fortification is to identify a bioavailable iron fortification compound which does not induce unacceptable sensory changes in the selected food vehicles. In general, highly water-soluble iron fortification compounds cause unacceptable sensory changes in food vehicles, whereas poorly soluble iron fortification compounds do not react with the food matrix but are less bioavailable (Hurrell, 2002a).

2.6.1 Colour and flavour changes

The most visible effects of soluble iron compounds on the fortified food is its change in colour (Mellican *et al.,* 2003) and flavour (Bovell-Benjamin *et al.,* 1999). Ferrous sulfate rapidly discolours in fortified salt (Wegmuller, Zimmermann and Hurrell, 2003), it turns fortified extruded grains brown (Kapanidis and Lee, 1996) and reacts with a range of other foods, as reported in bananas (Hurrell, 1999), gingerbread, wheat flour (Hallberg *et al.,* 1989), infant foods (Hurrell, Furniss, Burri, Whittaker, Lynch and Cook, 1989), chocolate drinks (Hurrell, Reddy, Dassenko and Cook, 1991) and milk (Gaucheron, 2000). NaFeEDTA and reduced iron had negative effects on colour of fortified extruded rice (Moretti *et al.*, 2005). Van den Wijngaart and Codling, (2013), observed in Indonesia that, flour fortified with NaFeEDTA produced noodles with a slight darker colour when compared to the noodles produced from unfortified (control) flour. Viteri, Xunian, Tolomei and Martin, (1995b) demonstrated that iron fortified maize porridge develops undesirable colour.

When sugar that was fortified with ferric EDTA was added to tea, the beverage turned black (Viteri *et al.,* 1995a). Iron is a transition metal, thus it participates in oxidation/reduction reactions (Mellican *et al.*, 2003). The iron induced discolouration in foods can be either due to the direct reaction of soluble ferric iron with oxygen to form iron oxides or to the reaction with other food components resulting in the formation of colour active substances (Mellican *et al.*, 2003).

Polyphenols have been reported to contribute to the colour development in foods fortified with soluble iron (Brune *et al.,* 1989). Hurrell (1997) suggested that polyphenols may be involved in the off-colour development of iron fortified foods. Bradshaw, Prenzler, and Scollary (2001) suggest that metal ions may interact with polyphenols in wine, causing browning in wine. Only certain structures of polyphenols seem to interact with iron to form discolouration. A study done in model solutions and in foods rich of polyphenols confirmed this finding by showing that ortho-hydroxy groups as found in gallic acid (e.g. in chocolate), catechin (found in green tea), chlorogenic acid (in coffee) cause off-colour developments with iron (Mellican *et al.*, 2003). The interaction of iron with polyphenols is an oxidation/reduction reaction, where the ferric (Fe^{3+}) iron is reduced to ferrous (Fe^{2+}) and the polyphenol compounds are oxidized (Mellican *et al.*, 2003). Additionally, fruits and vegetables contain polymerized polyphenols of high molecular weight, likely to include many ortho-hydroxy groups (Mellican *et al.*, 2003).

The neutral pH in the food matrix and the presence of oxygen can accelerate the oxidation of iron to its ferric form, which serves as a substrate in the colour formation reaction with polyphenols. Lower pH and the addition of reducing and chelating agents (ascorbic acid or EDTA) stabilized the ferrous ions reducing or inhibiting the colour formation. It has been suggested that the oxidation/reduction reaction that oxidizes polyphenols and reduces ferric iron back to its ferrous form might induce structural changes and polymerization in the polyphenols influencing their light absorption pattern (Mellican *et al.*, 2003).

The redox activity of soluble iron ions does not affect only the colour of the food. Soluble iron itself can have metallic or astringent taste, especially in beverages (Hurrell, 1999). In cereal flours, iron can catalyse the oxidation of unsaturated fatty acids and accelerate the formation of rancidity. Oxidation products such as hexanal or pentane can be determined to investigate the extent of oxidation occurred (Bovell-Benjamin *et al.*, 1999; Hurrell *et al.*, 1989). According to Hurrell *et al.*, (2000), iron fortified infant cereals have developed rancid flavours during storage and this was attributed to iron catalysed oxidation reactions. Since many flavours are lipids, iron can catalyse degradation of these susceptible compounds as well, leading to loss of desired flavours and formation of off-flavours. In milk, soluble iron compounds induce unpleasant odours and rancidity and increase the TBA number (thiobarbituric acid test), an oxidation marker which in milk products has been reported to correlate well with sensory evaluation (Gaucheron, 2000). Homogenisation, deaeration or pasteurisation at more than 81 ^{o}C have been suggested to reduce milk off-flavour or metallic taste (Gaucheron, 2000).

2.7 Iron fortification of special maize meal

Fortification is defined as the addition of nutrients based on nutritional needs, whereas enrichment restores the original nutrient content present, for example in rice grains before milling (Hoffpauer, 1992). Food fortification of staple foods with micronutrients is one of the food based strategies employed to alleviate micronutrient deficiencies in a population (WHO, 2001). In 2003, the Department of Health of South Africa introduced mandatory fortification of maize meal with electrolytic iron (at a minimum concentration of 35 mg iron/kg for special and super maize meal) in an attempt to combat ID. Maize meal was identified during a national food consumption survey (National Food Consumption Survey, 2000) as one of the most consumed (staple) foods products, thereby reaching lower income consumers most vulnerable to micronutrient malnutrition. ID was identified as being one of the major serious health risk factors in children aged between 1 - 9 years and women of childbearing age. These findings were used as motivation for the introduction of mandatory fortification in South Africa. Because of its low reactivity with food matrices and its low price, electrolytic iron is often chosen for fortification of cereal flours (Hurrell *et al.*, 2002). However, although electrolytic iron is inexpensive, its bioavailability is questionable because it can bind to phytates in cereals (INACG, 1993). In such cases, NaFeEDTA might be a better fortificant than electrolytic iron for supplementation of iron, because EDTA chelates iron, and might prevent it from binding to phytates. Isotope studies suggest that iron absorption from

NaFeEDTA might be two to three times higher than from electrolytic iron (Hurrell, 2002b). The high content of iron absorption inhibitors in maize meal, however presents special challenges. Thus an iron fortificant must be used that does not cause sensory changes and can supply iron despite the presence of phytates. NaFeEDTA has been shown to have high potential of being such a fortificant. Its ability to improve the bioavailability of both fortificant and intrinsic iron has been shown in isotopic studies (MacPhail, Patel, Bothwell and Lamparelli, 1994).

2.8 Maize meal processing

2.8.1 Maize

Maize *(Zea mays* L) is the staple food of many African countries including South Africa and many types of maize are grown around the world. It is an annual plant belonging to the grass family and it is a warm season crop requiring warmer growing temperatures than the small grains (for example wheat). In Africa, South Africa is one of the biggest producer of maize with an annual production of approximately 10 million tonnes, but depending on the rainfall, it can vary from as little as 2.9 million tonnes in the 1991/92 season (a severe drought year) to as high as 14.4 million tonnes in the 1980/81 season (Department of Agriculture, Forestry and Fisheries, South Africa, 2013). The 2012/13 season production was 11.7 million tonnes. Of the production, an average of 3.2 million tonnes is milled in the dry milling industry. The milled products are mainly used for human consumption with maize meal (super and special maize meal) being the largest products (Department of Agriculture, Forestry and Fisheries, South Africa, 2013).

2.8.2 Structure and mineral composition of the maize kernel

The maize kernel is the largest of the cereal grains, with an average kernel weight of 300 mg. A typical maize kernel is composed of 70 - 75 % starch, 8 - 10 % protein, 4 - 5 % lipid, 1 - 3 % sugars and 1 - 4 % ash (Tenaillon and Charcosset, 2011). The maize kernel components consist of an endosperm, germ, a pericarp and tip cap (Figure 2.2).

Maize mineral content ranges from 1.0 to 1.3 %. The germ alone provides nearly 80 % of the kernel's minerals, compared to less than 1 % from the endosperm. Phosphorus (in the form of phytate) (0.29 % dry basis), K (0.37 % dry basis) and Mg (0.14 % dry basis) are the most prevalent minerals found in maize providing nearly 85 % of kernel mineral content (Watson, 2003). As with most cereal grains, maize is low in Ca (0.03 % dry basis) and Fe (30 µg/g) or

3 mg/ 100g or 30 ppm. The bioavailability of Ca and Fe can also be retarded by the phytate concentrated in the maize germ (Bohn, Meyer and Rasmussen, 2008; Arendt and Zannini, 2013).

Genetic and environmental factors (soil quality, growing altitudes) have substantial impacts on kernel Fe and Zn contents. In this regard, Oikeh, Menkir, Maziya-Dixon, Welch and Glahn (2003) evaluated the concentrations of these minerals in the grains of elite-maturing maize varieties grown in diverse environments and examined their bioavailability using an *in vitro* digestion/Caco-2 cell model. They found that environment did not have a significant effect on kernel Fe and Zn levels provided the minimum growth requirements are met; however genotypic variation between cultivars had a highly significant impact. The genetic component accounted for 12 % of the total variation in kernel-Fe and 29 % for kernel-Zn levels. Maize kernel-Fe concentrations have been reported to range between 9.6 and 63.2 (mg/kg) and maize kernel-Zn have been reported to range between 16.5 to 24.6 (mg/kg). These authors also highlighted how genetic differences in the kernel also influence Fe bioavailability.

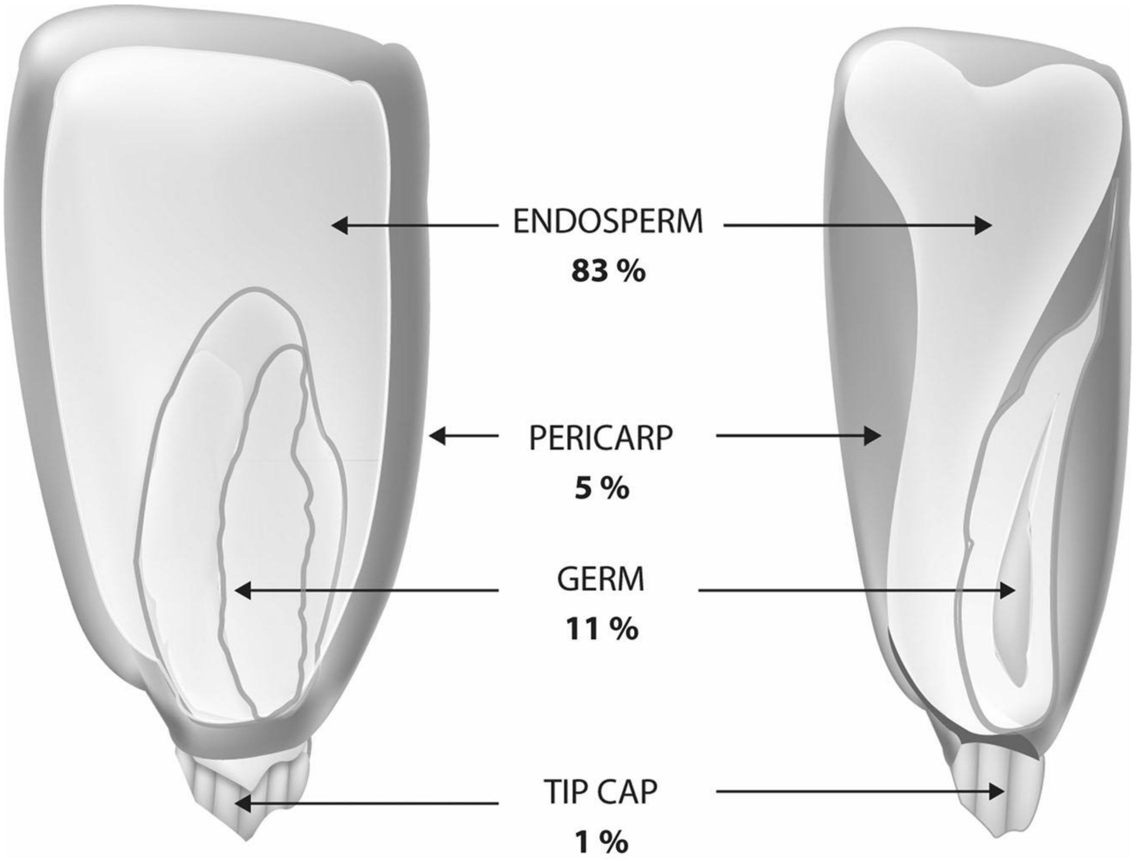


Figure 2.2: Components of maize kernel (Gwirtz and Garcia-Casal, 2014)

2.8.3 Full-fat dry milling

The full-fat milling process, also known as whole kernel dry milling, is the oldest maize milling process. The full-fat milling process yields products that contain most of the maize oil which is naturally found in the maize germ (typically contains 34 % lipids), as it grinds the maize kernel into uniform particles as opposed to fractionation. This product has a relatively short shelf-life because of the high fat content and endogenous enzymatic activities that can potentially cause rancidity and off-flavours (Hammond and Jez, 2011).

2.8.4 Maize dry milling

Dry milling is a process that is able to separate grain components by grinding maize into various particle sizes through the use of roller mills (Vanara, Reyneri and Blandino, 2009). Different maize meal products that differ in their extraction rate are produced using a dry milling process for human consumption in South Africa. Figure 2.3 shows a process line in the production of maize meal. After the cleaning step, which includes passage under a magnet to remove metal, aspiration to remove fine pieces of cob, and screening to separate the broken kernel from the whole kernel, maize is tempered to about 20 % moisture in a tempering bin. Tempering is mainly to generate differential swelling resulting from the germ and pericarp absorbing moisture and swelling faster than the endosperm. The differential swelling properties between the pericarp and the aleuerone layer of the endosperm, and between the germ and the endosperm, facilitate the further separation of the different maize components. The tempered maize kernels are then processed in the degerminator which, through abrasive action, removes the germ and the bran from the endosperm, leaving the latter intact. Different categories of de-germinators are available, such as Beall type, impact type, multiple impact/share or compression and roller milling, each one with its own particular characteristics and performances (Arendt and Zannini, 2013).

The products obtained from the tempering-de-germing process are maize grits, maize meal, and maize flour which are obtained as a result of particle size reduction on the roller mills (Johnson, 2000).

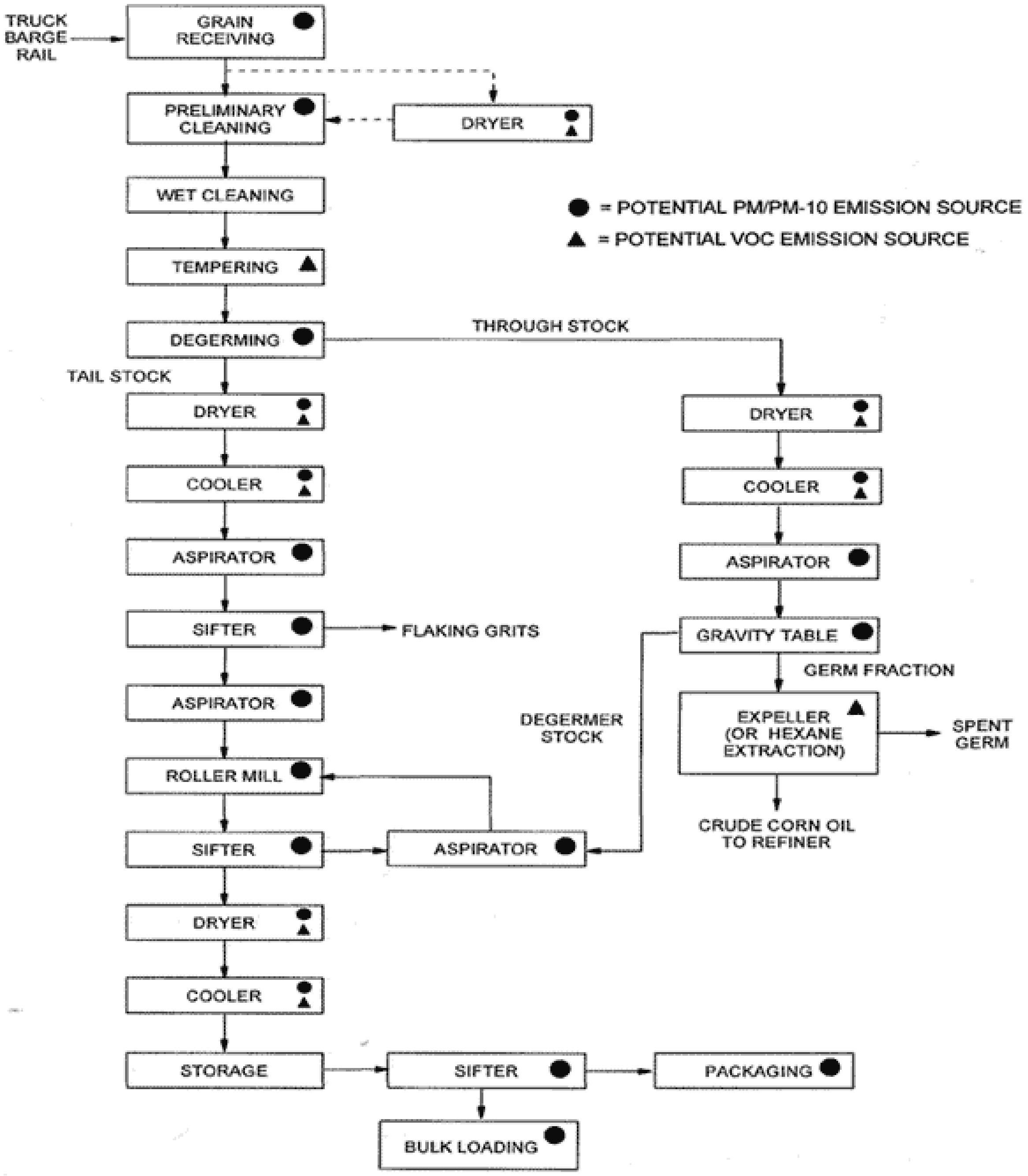


Figure 2.3: Simplified process flow diagram of a typical flour mill (Arendt and Zannini, 2013)

2.8.5 Maize products and food uses

The milled maize products intended for direct human consumption may be made from white or yellow maize and may differ in particle size, degree of extraction and whether or not the germ has been removed. Extraction rate refers to the weight of flour produced or extracted from the grain compared to the weight of the original wheat grain, expressed as a percentage (Nalubola and Nestel, 2000). According to the arbitrary split between low and high extraction specified in the WHO guidelines, 80 % extraction rate is considered the norm worldwide in the flour milling industry. The actual proportion of endosperm to the total maize kernel is about 80 %, but typical extraction rates run between 63 % and 79 % (Randall, Johnson and Verster, 2012).

Table 2.2 shows the composition of maize meals available in South Africa. The maize meal is normally consumed as stiff maize porridge. All but unsifted products are intended to be fortified (Johnson, Manaar and Ranum, 2004). Maize products for human consumption can be generally classified into three product categories in South Africa - super, special and sifted. Super maize meal (with low extraction rate and very high starch content) has the finest particle sizing, a fat content of less than 1.5 %, virtual total separation of germ and is the whitest in colour. It has a phytic acid content of 0.26 g/100g. It also sells at a premium price due to consumer preference for white, ‘fluffy’ easy-to-cook maize meal made possible by the low fat content of the product. In high technology roller mills such as those used by large milling companies in South Africa, it is possible to obtain an extraction rate of 60 - 65 % super. Although special maize meal (intermediate extraction rate) is not considered to be as desirable as super because of its higher fat content of 3.0 % (which tends to hamper easy cooking) and more yellow appearance when cooked. Special maize meal has an extraction rate of 79 %. It has phytic acid content of 0.25 g/100g. It is available at a comparatively lower price and still carries a large demand within lower socio-economic market sectors. Sifted (which includes the whole maize germ, a fat content from 4 - 5 % and is the equivalent of a whole crushed kernel) is generally not perceived as a suitable milled finished product, carrying relatively little independent returns within South Africa. Sifted maize meal has got a very high extraction rate (89 %) and low starch content (Higgins, 2010).

Table 2.2: Maize meals produced in South Africa (Johnson *et al.*, 2004)

Maize Meal Product Name	Ash content target	Fat target	Extraction target	Percentage of market
Super	0.55 %	1.5 %	63 %	36 %
Special	0.85 %	3.0 %	79 %	36 %
Sifted	1.10 %	3.7 %	89 %	12 %
Unsifted	------	-----	~100 %	-----

2.9 Types of different cooking vessels

Aluminium (Al) and cast iron cookware are typically used for preparing food in rural homes (Verissimo, Oliveira and Gomez, 2006). Al cookware if often preferred because of its low price while cast iron cookware is preferred because of its durability (Prinsen Geerligs, Brabin, Mkumbwa, Broadhead and Cuevas, 2002). Stainless steel cookware is widely used in food preparation in urban homes and also in commercial cookware (Kuligowski and Halperin, 1992).

2.9.1 Physical and chemical properties of metals and metal alloys used for cooking vessels

2.9.1.1 Physical properties

Different metals and metal alloys differ in thermal conductivity and heat transfer. Thermal conductivity of a material is the heat flow from a hot to a cold region. The temperature gradient between the hot and cold ends is a measure of the thermal conductivity of the material. Its unit of measurement is watts per meter Kelvin (W $m^{-1}K^{-1}$). Alloys have lower thermal conductivities than pure metals. Aluminium cookware is made from only pure aluminium metal. Stainless steel cookware is an alloy of mainly iron, chromium and carbon while cast iron cookware is an alloy of mainly iron, silicon and carbon. Stainless steel has a thermal conductivity of 16 W $m^{-1}K^{-1}$, cast iron has a thermal conductivity of 58 W $m^{-1}K^{-1}$and aluminium has a thermal conductivity of 225 W $m^{-1}K^{-1}$ (Tilley, 2004).

2.9.1.2 Chemical properties

Studies on different types of commonly used cookware have demonstrated that different cookware does leach metal ions (mainly iron and aluminium) into the food during cooking process (Bi, 1995; Kuligowski and Halperin, 1992; Verissimo *et al.,* 2006).

2.9.1.2.1 Chemical interactions of Aluminium (Al) with water (Aluminium pot)

Aluminum is not an essential element to humans, and is considered to be a toxic metal ion. Al pans are the most commonly used cooking utensils in rural places (Prinsen Geerligs *et al.,* 2002) and there has been some evidence that Al does leach into the food product during cooking (Verissimo *et al.,* 2006). Aluminium leaching from cookware is one of the important sources of Al that is ingested by humans. This leaching process is highly pH dependent and also dependent on the presence of complexing species. It is suggested that in the pH range of most foods (pH 4-8) Al present is predominantly in the form of organic Al-complexes, which is harmful to the human body (Verissimo *et al.,* 2006). The leaching process can be explained by the following chemical reaction that occurs on the surface of the aluminium utensils in contact with water:

$$Al_2O_3 + 6H^+ = 2Al^{3+}\ 3H_2O$$

where Al_2O_3 is a protective film. The free aluminium (Al^{3+}) in solutions reacts with organic acids found in food, like citric, oxalic, acetic and other complexing ligands like fluoride ion hydroxyl. These reactions may take place simultaneously and promote each other (Bi, 1995).

2.9.1.2.2 Stainless steel cookware

Stainless steels are widely used materials in food preparation in home as well as in commercial cookware (Kuligowski and Halperin, 1992). Stainless steels of various compositions are widely sold and used as home cookware. "Stainless steel" is a phrase used to mean any material which is mainly iron and contains more than 11 % chromium (Cr). The carbon (C) content is carefully controlled (e.g. ranges from 0.006 % to 0.08 %). The composition of stainless steel varies from 50 to 88 % Fe, 11 to 30 % Cr, 0 to 31 % Nickel and 0.006 % to 0.08 % C. Various other elements are present in minor amounts. Most commercially used stainless steel contain nickel (Ni) in amounts greater than 8 %; in food-handling the most widely used stainless steel contain 18 % Cr, 8 % Ni, and 70 to 73 % Fe (Kuligowski and Halperin, 1992). Stainless steel is readily attacked by organic acids, particularly at high cooking temperatures; hence iron, chromium and nickel should be released from the material into the food. Cooking food products of pH range 1.8 to 6.0 does contribute to the corrosion of stainless steel cookware. When 5 % acetic acid was boiled for 5 minutes, the corrosion of stainless steel utensils, measured by the quantity of iron in the water, ranged from less than 0.28 mg/kg to 2.9 mg/kg (Kuligowski and Halperin, 1992).

2.9.1.2 .3 Cast iron cookware

Cast iron is typically mostly iron (88 to 92 %), 3 to 5.5 % carbon, 1 to 2 % silicon and small amounts of other elements (magnesium 0.91 %, phosphorus 0.598 %, sulfur 0.149 %, potassium 0.59 %). The quantity of ingested iron from cooking acidic food prepared in cast iron pot is considerable. When 5 % acetic acid was boiled for 5 minutes, the corrosion of cast iron utensil, measured by the quantity of iron in the water was observed to be 3.5 mg/kg (Kuligowski and Halperin, 1992). The use of cast iron pots has been discussed as a potentially low cost intervention to counteract iron deficiency. In a randomized trial, the use of cast iron pots for cooking has been shown to improve iron status, haemoglobin and linear growth after six months of use (Adish, Esrey, Gyorkos, Jean-Baptiste and Rojhani, 1999). The food consumed from cast iron pots contained approximately double the amount of iron than the food cooked in aluminum pots, in preliminary laboratory tests (Adish *et al.*, 1999). In a study in rural Malawi however, the acceptability of the use of a cast iron pot was significantly lower than for an aluminum pots, and the main complaints were the high weight and the rusting surface of the cast iron pot (Prinsen Geerligs *et al.*, 2002). Acceptability and the sustainability might therefore be limiting factors for the use of non-steel cast iron pots in iron deficient communities.

2.10 Gaps in knowledge

This review shows that NaFeEDTA can potentially be used to replace electrolytic iron in the South African maize meal national fortification programme. NaFeEDTA is the only iron fortificant compound recommended by the WHO to be used in high extraction cereal flours (maize and wheat). High extraction cereal flours have high content of iron inhibitors (phytic acid and polyphenols). In high phytate diets, which predominate in the developing countries, the absorption of iron from NaFeEDTA is 2 - 3 times the absorption from ferrous sulfate. Ferrous sulfate is used as reference to assess the relative bioavailability of other iron fortificants, thus it is used as the standard for means of comparison.

NaFeEDTA can potentially be used in the fortification of maize meal in South Africa. The compound has the potential to reduce the prevalence of iron deficiency and iron deficiency anaemia. However, there is no information on the possible sensory effect of NaFeEDTA-fortified maize meal evaluated as stiff maize porridge and thus warranting research to be done. Furthermore, the type of cooking material (cast iron, stainless steel or aluminium cookware) has been hypothesised to potentially contribute to the possible negative sensory problems hence this investigation needed to be done.

CHAPTER 3: HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

The replacement of electrolytic iron with NaFeEDTA as part of a standard multi-nutrient fortification mix, for maize meal will lead to darkening of the colour (L* a* b*) and change in appearance (sensory evaluation) of the maize meal and prepared stiff porridge respectively. The effect will be dose dependent. The colour will darken even more when the stiff porridge is stored for 24 hours. According to Pozo-Insfran *et al.,* (2006) white maize contains polyphenolics such as ferulic and p-coumaric acid along with their respective derivatives. These polyphenols can interact with iron ions that are added to the maize meal. The interaction of iron with polyphenols is an oxidation/reduction reaction, where the ferric (Fe^{3+}) iron is reduced ($Fe^{3+} + e^{-} \rightarrow Fe^{2+}$) and the polyphenol compounds are oxidized (Mellican *et al.,* 2003). These reactions lead to the formation of off-colour in food (Mellican *et al.,* 2003; Hurrell, 1997).

The replacement of electrolytic iron with NaFeEDTA as part of a standard multi-nutrient fortification mix for maize meal will promote lipid oxidation and development of flavour changes of stiff maize meal porridge which will be dose dependent. The flavour changes will even be more severe when the stiff porridge is stored for 24 hours. Maize contains polyunsaturated fatty acids (PUFAs) in the form of linoleic acid (Bovell-Benjamin, *et al.,* 1999). Iron ions (Fe^{2+}/Fe^{3+}) have the capacity to promote destructive free-radical reactions, which can result in the development of off-flavours. Thus the addition of iron ions into fat (mainly polyunsaturated fatty acids) containing products causes flavour changes due to lipid oxidation (Rekhif *et al.*, 2002; Mellican *et al.*, 2003).

Preparation of stiff porridge from maize meal fortified with electrolytic iron or NaFeEDTA in aluminium or cast iron pots will lead to increased darkening compared porridge prepared in stainless steel pots. According to Mellican *et al.,* 2003 darkening of stiff maize meal porridge is due to the oxidation of polyphenol compounds (ferulic and p-coumaric), a reaction that will be enhanced in an environment where Al^{3+} is reduced ($Al^{3+} + 3e^{-} \rightarrow Al$). The possible interaction of aluminium with polyphenols is an oxidation/reduction reaction. In the case of cast iron pots, there is evidence that considerable amount of iron leaches into food during cooking (Prinsen Geerligs, Brabin and Omari, 2003). The leached iron has the potential to undergo oxidation/reduction reactions with polyphenols found in maize and thus cause darkening of porridge.

3.2 Objectives

The following objectives will be addressed according to the experimental design shown in Fig. 3.1.1

Phase 1: Sensory evaluation

To determine the effect of replacing electrolytic iron with nutritionally comparable levels of NaFeEDTA (15 mg iron /kg and 30 mg iron /kg) on the colour (L* a* b*) and sensory properties (appearance and flavour) of stiff maize meal porridge prepared in stainless steel pots, cast iron pots and that prepared in aluminium pots, that has been freshly prepared and stored for a time period of 24 hours at a temperature of 3 - 5 ^{o}C.

Phase 2: Consumer sensory evaluation

Should the replacement of electrolytic iron with NaFeEDTA have any effect on the sensory properties of stiff porridge when judged by the trained panel, then the effect of the change on consumer acceptance of porridge will be determined.

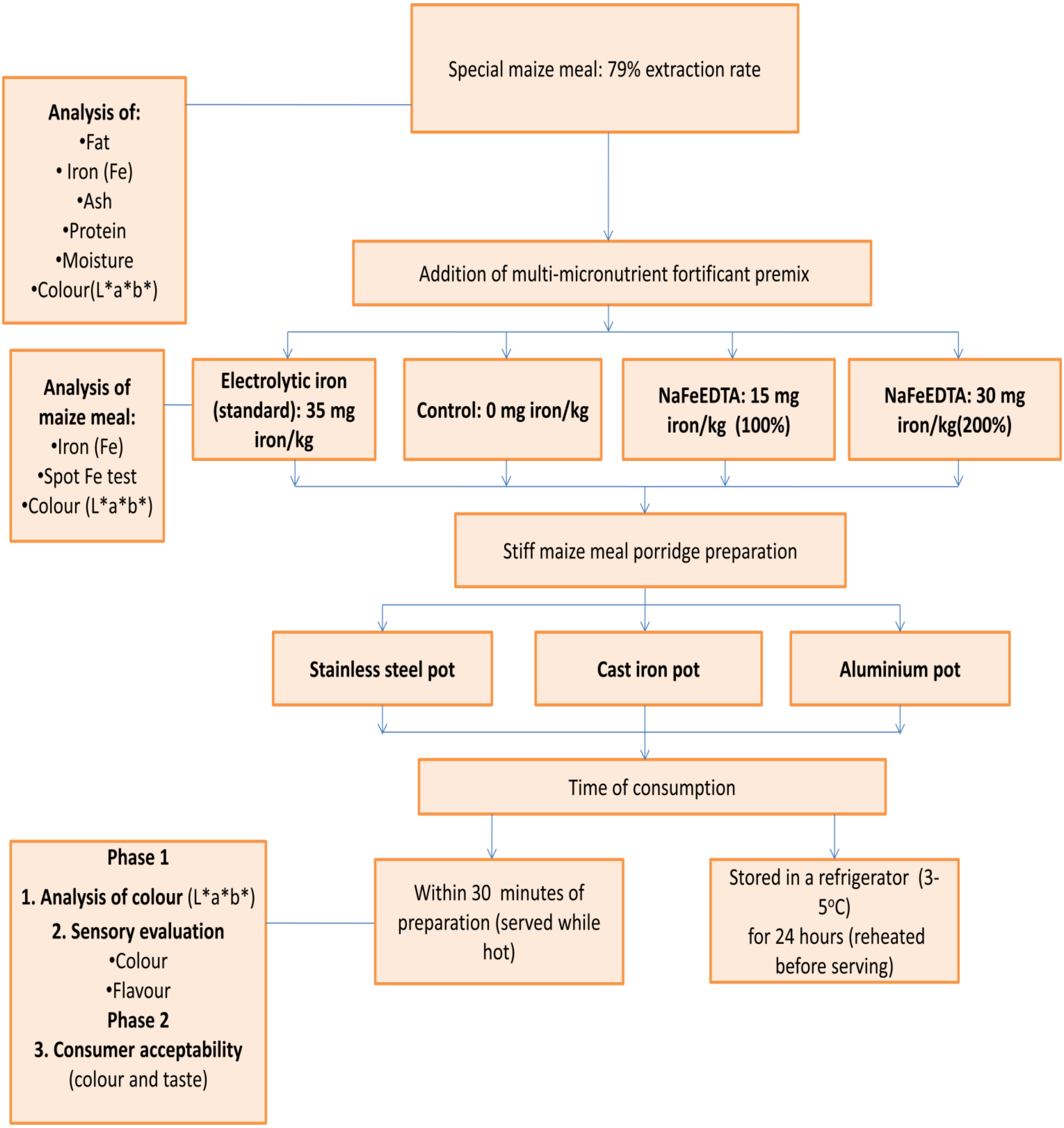


Figure 3.1.1: Experimental design of the research

CHAPTER 4: RESEARCH CHAPTER

4.1 The effect of replacing electrolytic iron, in a multi-micronutrient fortification mix, with sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) on the sensory properties of porridge from maize meal

Abstract

Iron deficiency has been classified as the most prevalent micronutrient deficiency in the world. Inadequate iron intake leads to anaemia in young children, adolescents and women, most of whom live in developing countries. Iron fortification of food is regarded as the most cost-effective method for reducing the prevalence of iron deficiency. However, the iron compounds used may lead to undesirable sensory changes, especially colour and flavour in the foods being fortified. This study evaluated the effects of replacing electrolytic iron (35 mg iron/kg maize meal) in a multi-micronutrient fortificant premix with NaFeEDTA (15 and 30 mg iron/kg maize meal) added to special maize meal on the colour (L* a* b*) and sensory properties (appearance and flavour) of stiff porridges. The porridges were prepared using three different types of cooking vessels (stainless steel, cast iron and aluminium pots) and was evaluated fresh (within 30 minutes of preparation) and after 24 hour refrigerated storage (3 - 5 ^{o}C). Control samples were prepared with all micronutrients added except iron. All iron-fortified maize meals and maize porridges had significantly lower L* values than control samples. The type of cooking vessel used did affect the appearance and flavour of the maize porridge. However, considering human perception of the porridges, it appears that fortifying maize meal with NaFeEDTA at 15 mg iron/kg maize meal should not lead to changes in appearance and flavour of special maize porridge compared to what consumers are currently used to. Even an excess amount of NaFeEDTA at 30 mg iron/kg maize meal was not detected as a change. The more bioavailable form of iron (NaFeEDTA) could be a viable option to reduce prevalence of iron deficiency in developing countries.

4.1.1 Introduction

Iron deficiency is the most common nutritional deficiency in the world and affects mostly infants, children, and women of childbearing age (FAO, 2004; Van Stuijvenberg, Smuts, Lombard and Dhansay, 2008). Fortification of maize meal, a staple food with iron, is a preventive food-based approach to improve iron status of populations (Randall *et al.*, 2012). South Africa has mandatory fortification of maize meal where electrolytic iron forms part of the legislated multi-micronutrient fortificant premix (Department of Health South Africa, 2003). A proposal to change from electrolytic iron to NaFeEDTA, a more bioavailable form of iron, has been put forward by the Department of Health, South Africa (Personal communication, M. Hoop, Department of Health, South Africa, 2014).

The need for iron fortification is well-established; however, technical challenges for fortifying foods with iron still exist (Mellican *et al.,* 2003). The dilemma is that iron compounds that are water soluble and highly bio-available, e.g. ferrous sulfate, may cause undesirable colour and flavour changes in the food to which it is added, whereas compounds that are less soluble and therefore more stable in foods (e.g. electrolytic iron powders) are poorly absorbed (Hurrell, 2002a). For time saving purposes, stiff maize porridge is often prepared in large quantities and some of it is stored for consumption later e.g. the next day. Concerns, based on anecdotal evidence, were raised that porridge fortified with iron tends to develop undesirable colour and flavour changes when stored overnight. It was suggested that such colour changes might be linked to the iron fortificant compound, in this case, electrolytic iron used in the South African maize meal fortification programme. The severity of the colour changes is hypothesised to be dependent on the type of cookware used.

This study evaluated the effects of replacing electrolytic iron (35 mg iron/kg maize meal) in a multi-micronutrient fortificant premix with NaFeEDTA (15 or 30 mg iron/kg maize meal) added to special maize meal on the colour (L* a* b*) and sensory properties of stiff porridges. The porridges were prepared using three different types of cooking vessels (stainless steel, cast iron and aluminium pots) and was evaluated fresh (within 30 minutes of preparation) and after 24 hour refrigerated storage (3 - 5 ^{o}C).

4.1.2 Materials and Methods

4.1.2.1 Materials

Unfortified special maize meal (79 % extraction rate) was obtained from Premier Milling (Pretoria, South Africa). Multi-micronutrient fortificant premixes (Table 4.1.1) were prepared by DSM Nutritional Products South Africa (Pty) Ltd. (Isando, South Africa). Three fortificant premixes were supplied containing electrolytic iron (IS-353) or NaFeEDTA (IS-1056) and a control premix with no iron added (IS-1056 excluding iron).

4.1.2.2 Mixing of special maize meal with fortificant premixes

The mixing of the multi-micronutrient fortificant premix and maize meal was done at the Southern African Grain Laboratory (SAGL) following internal accredited standard procedures. In the case of electrolytic iron (IS-353), a premix dosage rate of 200 mg/kg was used with the aim of a final maize meal concentration of 35 mg iron/kg maize meal. This fortificant premix (IS-353) was used as a standard since electrolytic iron is currently being used in the maize meal national fortification programme in South Africa (Department of Health, South Africa, 2003). In the case of NaFeEDTA (IS-1056), to obtain the different levels of NaFeEDTA, the quantity of the premix added was altered in the following manner. A premix dosage rate of 300 mg/kg was used with the aim of a final maize meal concentration of 15 mg iron/kg maize meal and this was considered as 100 % compliance with proposed fortification regulations (Personal communication, M. Hoop, Department of Health, South Africa, 2014). The dosage rate was also increased (200 %) to 600 mg/kg with the aim of a final maize meal concentration of 30 mg iron/kg maize meal. Maize meal fortified with fortificant premix (IS-1056 excluding iron) containing 0 mg iron/kg maize meal was added at a dosage rate of 300 mg/kg and was used as a control.

Table 4.1.1: Special maize meal multi-micronutrient fortificant premixes containing electrolytic iron, NaFeEDTA or no iron

		Micronutrient concentration (mg/kg maize meal)			**Multi-micronutrient fortification premix by compound (g/kg)**		
Micronutrient	**Fortificant compound**	Premix with NaFeEDTA **(IS - 1056)**	Premix excluding iron **(IS - 1056 excluding iron)**	Premix with electrolytic iron **(IS - 353)**	Premix with NaFeEDTA **(IS - 1056) (g/kg)**	Premix excluding iron **(IS - 1056 excluding iron) (g/kg)**	Premix with electrolytic iron **(IS - 353) (g/kg)**
Vitamin A	Retinyl Palmitate	2.10	2.10	2.09	93.33	93.33	139.00
Vitamin B_1	Thiamine mononitrate	2.19	2.19	2.19	9.01	9.01	14.02
Vitamin B_2	Riboflavin	1.69	1.69	1.69	5.63	5.63	8.44
Vitamin B_3	Niacinamide	25.00	25.00	25.00	84.18	84.18	125.00
Vitamin B_6	Pyridoxine hydrochloride	3.13	3.13	3.13	12.72	12.72	19.29
Vitamin B_9	Folic acid	2.00	2.00	2.00	7.14	7.14	11.05
Vitamin B_{12}	Cobalamin	0.005	0.005	-	16.670	16.670	-
NaFeEDTA	NaFeEDTA	15.00 (as iron)	-	-	384.62	-	-
Electrolytic Fe	Electrolytic Fe	-	-	35.00 (as iron)	-	-	178.67
Zinc	Zinc Oxide	30.00	30.00	15.00	125.00	125.00	93.75
Diluent	Diluent	-	-	-	261.43	261.43	410.78
(dosage rate g/MT)		300	300	200	300	300	200

NaFeEDTA, sodium iron (III) ethylenediaminetetraacetate. Premix prepared by Dutch State Mines (DSM), Johannesburg, South Africa.

4.1.3 Methods

4.1.3.1 Maize meal porridge preparation

Maize meal porridge was prepared according to a method by Kuyper, deKock and Jurgens, (2000) modified as follows: 1.0 L water and 5 g salt was heated to boiling point in a cooking pot, on a hot plate (Anvil, STA 0001, Pretoria, South Africa) set at level 4. A total of 450 g of maize meal was used as follows: 200 g maize meal was mixed with 500 ml cold water to make a slurry. The slurry was then added into the boiling water and thoroughly stirred using a wooden spoon and then allowed to cook for 10 minutes at level 3. After 10 minutes, the remaining maize meal (250 g) was added while thoroughly stirring with a wooden spoon. The stirring was thorough and consistent so as to avoid the development of lumps. The porridge was allowed to simmer (level 3) for 20 minutes while mixing the porridge every 10 minutes. The temperature was reduced to level 2 for the final 10 minutes, bringing the cooking process to 40 minutes. The pots were transferred onto pre-heated hot trays before serving to the trained sensory panel. The porridges were prepared in three different types of cooking pots; cast iron pots (3 liters in volume), stainless steel pots (3.2 liters in volume) and aluminium pots (6 liters in volume).

4.1.3.2 Colour analyses of maize meal and cooked maize porridge

Maize meal (30 g samples) and 40 g cooked maize porridge samples were placed in petri dishes and pressed flat. The L^*, a^*, b^* of each maize meal sample was determined in triplicate using a Minolta Chroma Meter CR-400/410, (Konica Minolta Sensing, Japan), calibrated with a white plate (CIE L* = 97.91, a* = - 0.68, b* = 2.45). CIELAB scale L*: 0 = black, 100 = white; a*: negative values indicate green, positive values indicate red; b*: negative values indicate blue, positive values indicate yellow (Richins, Burton, Pahulu, Jefferies and Dunn, 2008). For cooked porridges, the colour was measured twice, first, within 30 minutes of sample preparation and also after 24 hours of storage at 3 - 5 ^oC. The porridges were stored in glass ramekins covered with plastic lids. The difference in colour of freshly prepared maize porridge (expressed as ΔE) was evaluated with the parameter $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$ where (L_1 or a_1 or b_1 was for porridge with 0 mg iron/kg maize meal) while L_2 or a_2 or b_2 was for porridge fortified with NaFeEDTA - 15 mg iron/kg maize meal; NaFeEDTA - 30 mg iron/kg maize meal or electrolytic iron - 35 mg iron/kg maize meal respectively. Similar colour difference (ΔE) calculations were also done for the stored maize meal porridges.

4.1.3.3 Moisture analysis

Moisture content of the unfortified maize meal was determined using AOAC method 934.01 (AOAC, 2000a). The analysis was done in duplicate.

4.1.3.4 Crude protein analysis

Crude protein content of unfortified maize meal was determined using AOAC method 968.06 (AOAC, 2000b). The analysis was done in duplicate.

4.1.3.5 Crude fat analysis

Crude fat content of unfortified maize meal was determined using AOAC method 920.39 (AOAC, 2000c). The analysis was done in duplicate.

4.1.3.6 Ash content analysis

Ash content of unfortified maize meal was determined using AOAC method 942.05 (AOAC, 2000d). The analysis was done in duplicate.

4.1.3.7 Spot iron test for iron in maize meal (qualitative method)

The qualitative analysis of iron distribution in the different maize meal treatments was determined in triplicate using the spot iron test AACC Method 40-40 (AACC, 2000). The maize meal that had been subjected to the spot iron test in the petri dishes was also subjected to colour analysis using the colorimeter (Minolta Chroma Meter CR-400/410, Konica Minolta Sensing, Japan). Commercially available special maize meal, fortified with electrolytic iron at 35 mg iron/kg maize meal (as per current South Africa law and regulation) was purchased in a local store for comparison. Measurements were taken in triplicate.

4.1.3.8 Mineral (Fe, Zn, Ca) analysis of maize meal

Fe content of the maize meal treatments and Fe, Zn and Ca content of unfortified maize meal were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES) after microwave digestion (AOAC, 2000). The analysis was done in triplicate.

4.1.4 Sensory evaluation

4.1.4.1 Recruitment and screening of panellists

Interested persons within and outside the University of Pretoria were invited via email, social networks, posters and word of mouth to apply to be part of the analytical sensory panel. They had to meet criteria such as be available, have interest in the project, no food allergies, no chronic diseases (self reported) and normal sensory acuity before they were invited to participate.

About sixty people responded and attended the introduction session with some of them already being trained panellists. The trained panellists had to confirm their availability. The remaining untrained persons had to be screened for sensory acuity using various methods of screening such as identification of basic tastes (sweet, sour, salty, bitter and umami), identification of different aromas using smelling strips, and difference testing by triangle tests of breakfast cereals and of fizzy drinks. They were also provided with two maize porridge samples where they had to identify the sample which was stiffer. A final panel of 28 judges who scored the highest in the above mentioned activities were selected and used.

4.1.4.2 Training of the panel

Training of the panel involved introducing them to the Difference from Control (DFC) test method (Meigaard, Civille and Carr, 2007) and also explaining the reasons for conducting the sensory evaluation of the maize meal porridges. The DFC method is used when the test objective is twofold, (1) to determine whether a difference exists between one or more samples and a control, and (2) to estimate the size of any such difference. Generally one sample is designated the control and all the other samples are evaluated with respect to how different each is from the control (Lawless and Heymann, 2010).

4.1.4.3 Sample preparation, coding and evaluation

Five maize porridges (30 - 40 g) served at ± 52 ^{o}C in glass ramekin dishes, covered with plastic lids and then blind coded with either a three digit code or labeled as control (maize porridge fortified with multi-micronutrient premix excluding iron) were presented to each panellist per session. The porridges were presented either freshly prepared (within 30 minutes of preparation) or after storing for 24 hours at a temperature of 3 - 5 ^{o}C. In the case of the stored maize porridges, the samples were portioned into glass ramekins, covered with plastic lids and put on coded trays prior to sensory evaluation. The samples were reheated (one porridge sample at a time) in a 1100 Watts commercial microwave oven (Menumaster RCS511TS, Pretoria, South Africa), set for 30 seconds, to a temperature of ± 52 ^{o}C before being presented to the panellists.

Maize porridges were prepared in three different types of cooking pots (aluminium, stainless steel and cast iron pots). Twelve evaluation sessions (two per day) were held on six different days to evaluate maize porridges prepared in each type of cooking pot in duplicate. The order of sample presentation followed a William Latin Square design to minimise order carry over effects (Joeng, Jang, Chang and Lee, 2013). The sensory evaluation was conducted in the sensory evaluation laboratory with individual booths equipped with computers and Compusense five® release 5.2 (Compusense Inc, Guelph, ON, Canada) for direct data entry. White daylight was used in tasting booths. Five stored porridge samples were evaluated first while five freshly prepared porridges were evaluated after a 15 minutes break to avoid sensory fatigue. A total of 28 trained panelists (14 persons at a time) were presented simultaneously with five porridge samples. For each panellist, the control sample was always positioned first on the far left of the tray and then followed by four coded samples. The control sample was also presented as one of the blind coded samples and the panellists were alerted to this. The blind control helps to establish a base line for the rest of the test samples, as most blind controls will get a non-zero score due to individual variability (Lawless and Heymann, 2010). The five samples were presented on a white tray, with stainless steel teaspoons, a serviette and filtered tap water as a palate cleanser. Panellists were instructed to hold each coded sample next to the control, evaluating the overall appearance of each coded sample compared with the control, and then indicate if it was the same as the control or different; if different they had to rate the magnitude of the difference using a category scale (1 = same as control, 2 = slightly different from control, 3 = moderately different from control, 4 = very different from control and 5 = extremely different from control). The panellists then had to taste each coded sample against the control and rate the overall flavour of the maize porridges using the same category scale.

4.1.5 Statistical analysis

Data for each maize meal and maize porridge were expressed as mean ±standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the effect of different iron treatments on the colour (L*a*b*) of maize meal while factorial ANOVA was used to determine the effect of different iron treatments and storage time on the colour (L* a* b*) of maize porridges. For the results of difference-from-control sensory tests, two way ANOVA for each type of cooking pot (aluminium, cast iron and stainless steel pots) was separately used to determine the effect of iron treatment and storage time as main effects and also their interaction effect on appearance and flavour of porridges. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 22 (SPSS, Chicago, USA). Where significant effects were noted, Tukey Honestly Significant Difference HSD test at $p < 0.05$ was used to compare means.

4.1.6 Results

4.1.6.1 Special maize meal characterisation

The general nutrient composition of the unfortified maize meal is displayed in Table 4.1.2.

Table 4.1.2: General nutrient analyses of unfortified maize meal (as is basis)

Nutrient	Composition
Crude protein (g/100g)	6.50
Ash (g/100g)	0.60
Moisture (g/100g)	12.70
Crude fat (g/100g)	3.02
Ca (mg/kg)	52.50
Zn (mg/kg)	18.00

4.1.6.2 Iron content (mg/kg) of unfortified and fortified maize meals

The iron content of the fortified maize meals (Table 4.1.3) were slightly lower than expected.

Table 4.1.3: Iron content of unfortified and fortified maize meals

Maize meal treatment[a]	Iron (mg iron/kg maize meal, dry basis)
No fortification	7.65±1.51
Control (fortified with premix excluding iron)	10.05±2.68
NaFeEDTA15	21.20±2.02 [13.55]
NaFeEDTA30	32.78±1.24 [25.13]
Electrolytic iron	37.95±1.91 [30.30]

[] - values in square brackets are the fortificant iron in the maize meal (total iron content - intrinsic iron content, 7.65 mg iron/kg)

[a] Expected dosage with fortification was 15 mg iron/kg and 30 mg iron/kg for NaFeEDTA and 35 mg iron/kg for electrolytic iron for maize meals NaFeEDTA 15, NaFeEDTA 30 and electrolytic iron respectively

4.1.6.3 Colour values (L*a*b*) of maize meals

There was a general decrease in the L* values of maize meal with the addition of the micronutrient premix with or without NaFeEDTA or without electrolytic iron added (Table 4.1.4). The L*and a* values of maize meal with electrolytic iron were significantly ($p < 0.05$) lower than that of the control and that of maize meal with NaFeEDTA added. The L*, a* and b* values of maize meal with NaFeEDTA was not significantly ($p > 0.05$) different from the control.

Table 4.1.4: Mean (±SD) colour values (L* a* b*) of unfortified maize meal and maize meals fortified with electrolytic Fe or NaFeEDTA

Maize meal	L*	a*	b*
No fortification	90.95 ± 0.13^{c}	0.40 ± 0.03^{c}	8.45 ± 0.20^{c}
Control (fortified with premix excl.* iron)	89.28 ± 0.05^{b}	0.48 ± 0.01^{ab}	7.48 ± 0.14^{ab}
NaFeEDTA 15	89.23 ± 0.27^{b}	0.44 ± 0.04^{ab}	7.72 ± 0.38^{b}
NaFeEDTA 30	89.38 ± 0.14^{b}	0.50 ± 0.02^{a}	7.78 ± 0.01^{b}
Electrolytic Fe	88.69 ± 0.29^{a}	0.41 ± 0.05^{c}	7.15 ± 0.20^{a}

^{abc} = mean (±SD) values with different superscripts in the same column, differ significantly ($p < 0.05$)
*excl. = excluding

4.1.6.4 Qualitative spot test for iron distribution of unfortified maize meal and maize meals fortified with electrolytic iron or NaFeEDTA

Fewer red spots were observed in unfortified maize meal as compared to the iron fortified samples (Figure 4.1.1). The number of red spots reflects roughly the amount and distribution of the iron in the maize meal. There was a general increase in the number of red spots as iron concentration was increased in the maize meal. It was observed that the red spots formed by iron in the form of electrolytic iron were more visible when compared to spots formed by iron in the form of NaFeEDTA.

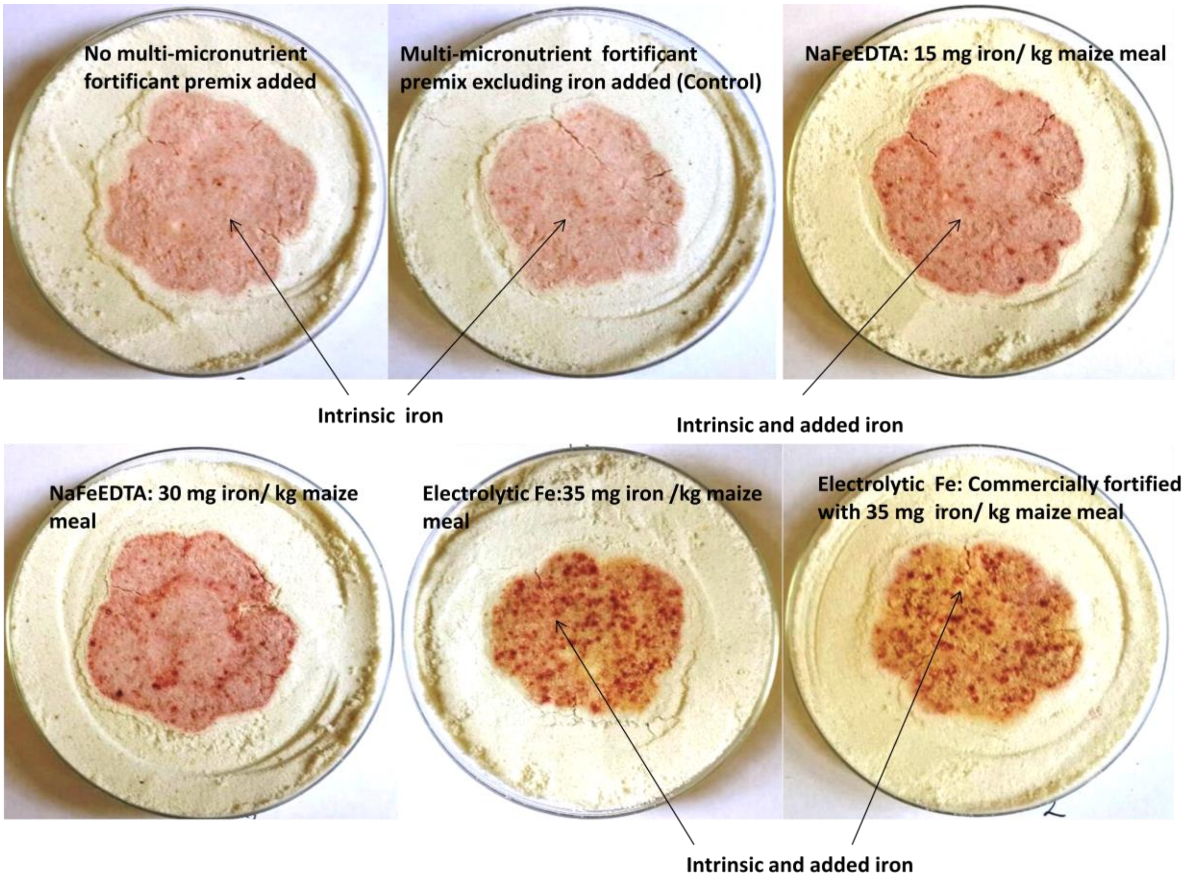


Figure 4.1.1: Spot iron test images of unfortified maize meal and maize meals fortified with electrolytic iron or NaFeEDTA

4.1.6.5: Colour values (L*a*b*) of unfortified maize meal and maize meals fortified with electrolytic iron or NaFeEDTA subjected to spot iron test

There was a significant decrease in L* values of maize meals subjected to the spot iron test chemicals with increase in iron concentration in maize meal (Table 4.1.5). Spot iron tested maize meals fortified with electrolytic iron, NaFeEDTA and that commercially fortified had significantly lower L* values to that of the control. There was significant ($p < 0.05$) increase in a* and b* values of the maize meals with increase in iron concentration in maize meal. NaFeEDTA 30 had the highest a* values (more red). The b* values of commercially fortified maize meal was significantly higher ($p < 0.05$) (more yellow) than the other fortified maize meals.

Table 4.1.5: Mean (±SD) colour values (L* a* b*) of maize meals subjected to spot iron (Fe) test

Maize meal	**L***	**a***	**b***
No fortification	75.53±1.51^{d}	9.34±0.52^{a}	9.26±0.69^{c}
Control (fortified with premix excl.* iron)	74.44±0.78^{d}	9.80±0.43^{a}	8.62±0.42^{b}
NaFeEDTA 15	72.67±0.61^{c}	10.97±0.49^{b}	7.56±0.27^{a}
NaFeEDTA 30	70.54±1.77^{b}	11.79±0.85^{c}	7.74±0.28^{a}
Commercially bought special maize meal	72.94±2.43^{c}	11.28±1.30^{bc}	13.56±0.65^{e}
Electrolytic iron	68.37±1.18^{a}	11.17±0.97^{bc}	12.20±0.73^{d}

^{abcde} = mean (±SD) values with different superscripts in the same column, differ significantly ($p < 0.05$)
excl.* = excluding

4.1.6.6: Colour values (L* a* b*) of freshly prepared (0 Hours) and stored (24 Hours) iron fortified maize meal porridges prepared in aluminium, cast iron and stainless steel pots

For porridges cooked in aluminium pots, there were no significant differences ($p > 0.05$) between the L*, a* and b* values of the NaFeEDTA 15 and 30, compared to the electrolytic iron fortified maize porridge (Table 4.1.6). For porridges cooked in aluminium pots, storage of maize porridge for 24 hours resulted in significantly increased L* ($p < 0.001$), and significantly reduced a* ($p < 0.001$), but not b* values ($p = 0.23$). For porridge cooked in aluminium pots, interaction between iron treatment and storage time was significant for a* ($p = 0.03$), but not L*and b* values. For fresh porridges, NaFeEDTA 15 had a difference in colour (ΔE) compared to control of 0.61 while ΔE for porridge fortified with electrolytic iron was 0.47. For stored porridges, NaFeEDTA 15 had a ΔE of 1.01 while porridge fortified with electrolytic iron had a ΔE of 0.91.

For porridges cooked in cast iron pots, there were no significant differences ($p > 0.05$) between L*and a*, but not b* values of the NaFeEDTA 15 compared to the electrolytic iron fortified porridge. There were significant differences ($p < 0.05$) between L* and b* but not a* values of the NaFeEDTA 30 compared to the electrolytic iron fortified porridge. For porridges cooked in cast iron pots, storage of maize porridge for 24 hours resulted in significantly higher L* ($p < 0.001$) and significantly lower b* ($p < 0.001$) values. For porridge cooked in cast iron pots, there was no significant interaction ($p > 0.05$) between iron treatment and storage time for L*, a*and b* values. For fresh porridges, NaFeEDTA 15 had a difference in colour (ΔE) compared to control of 0.81 while porridge fortified with electrolytic iron had a ΔE of 0.99. For stored porridges, NaFeEDTA 15 the ΔE was 0.40 while porridge fortified with electrolytic iron had a ΔE of 1.06 (Table 4.1.6).

For porridges cooked in stainless steel pots, there were no significant differences ($p > 0.05$) between L* and b* values of the NaFeEDTA 15 compared to the electrolytic iron fortified porridge but the a* values of NaFeEDTA 15 was higher ($p < 0.001$). There were no significant differences ($p > 0.05$) between L* and a* values of the NaFeEDTA 30 compared to the electrolytic iron fortified porridge but the b* value of NaFeEDTA 30 was higher ($p = 0.07$). For porridges cooked in stainless steel pots, storage of maize porridge for 24 hours resulted in higher L* ($p < 0.001$) and a* ($p < 0.001$) values, and lower b* ($p < 0.001$) values. For porridge cooked in stainless steel pots, interaction between iron treatment and storage time was significant for L*, but not a*and b* values. Fresh porridges, with NaFeEDTA 15 had a difference in colour (ΔE) compared to control of 0.64 while porridge fortified with electrolytic iron had a ΔE of 1.42. The ΔE was greater for stored than fresh porridges, NaFeEDTA 15 and NaFeEDTA 30 but surprisingly not for electrolytic iron (Table 4.1.6).

For cast iron pots all ΔE values for NaFeEDTA fortified porridges were lower for stored than for fresh prepared porridges implying that the colour of porridges were more similar to the control after storage for 24 hours while the opposite was found for porridges prepared in aluminium and stainless steel pots.

Table 4.1.6: Colour values (L* a* b*) of fresh (0 Hours) and stored (24 Hours) iron fortified maize meal porridges prepared in aluminium, cast iron and stainless steel pot

Maize meal porridge	**L* - values**			**a* - values**			**b* - values**			**ΔE to Control$^{\#}$ porridge**	
	Storage	**Time (ST)**	**Effect of Fe Treatment**	**Storage**	**Time (ST)**	**Effect of Fe Treatment**	**Storage**	**Time (ST)**	**Effect of Fe Treatment**	**Storage**	**Time**
Aluminium pot	**0 H**	**24 H**	(p = 0.01)	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**
Control$^{\#}$	71.61±0.17	74.62±0.55	73.12±1.69^{b}	-2.27±0.04	-2.37±0.07	-2.32±0.08^{a}	4.31±0.40	4.59±0.05	4.45±0.28^{c}		
NaFeEDTA15	71.77±0.25	73.86±0.61	72.82±1.22^{ab}	-2.02±0.04	-2.09±0.09	-2.06±0.07^{c}	3.78±0.28	3.99±0.29	3.89±0.28^{a}	0.61	1.01
NaFeEDTA30	71.00±0.13	73.48±0.69	72.24±1.43^{a}	-2.17±0.08	-2.29±0.05	-2.23±0.09^{ab}	4.41±0.12	4.29±0.12	4.35±0.13^{bc}	0.63	1.18
Electrolytic iron	71.52±0.16	73.91±0.23	72.71±1.32^{ab}	-2.00±0.06	-2.30±0.08	-2.15±0.18^{bc}	3.93±0.19	4.02±0.12	3.97±0.15^{ab}	0.47	0.91
Effect of storage time (ST)	71.47±0.34^{A}	73.97±0.64^{B}	**Fe x ST**	-2.11±0.12^{A}	-2.26±0.12^{B}	**Fe x ST^{1}**	4.11±0.35^{A}	4.22±0.29^{A}	**Fe x ST**		
	ST (p = 0.00)		(p = 0.30)	ST (p = 0.00)		(p = 0.03)	ST (p = 0.23)		(p = 0.47)		
Cast iron pot	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**
Control$^{\#}$	72.13±0.34	73.80±0.57	72.96±1.00^{b}	-2.02±0.07	-2.00±0.11	-2.01±0.09^{b}	4.38±0.30	4.04±0.30	4.21±0.33^{b}		
NaFeEDTA15	71.34±0.41	73.41±0.03	72.37±1.17^{a}	-1.89±0.05	-1.94±0.04	-1.92±0.05^{b}	4.28±0.23	3.98±0.14	4.13±0.24^{b}	0.81	0.40
NaFeEDTA30	72.02±0.29	74.01±0.48	73.02±1.15^{b}	-2.21±0.60	-2.14±0.07	-2.17±0.07^{a}	4.96±0.09	4.57±0.14	4.77±0.24^{c}	0.62	0.59
Electrolytic iron	71.38±0.25	72.95±0.04	72.16±0.87^{a}	-2.04±0.17	-2.04±0.02	-2.04±0.02^{ab}	3.74±0.39	3.40±0.10	3.57±0.31^{a}	0.99	1.06
Effect of storage time (ST)	71.72±0.47^{A}	73.54±0.53^{B}	**Fe x ST**	-2.04±0.14^{A}	-2.03±0.10^{A}	**Fe x ST**	4.34±0.51^{A}	4.00±0.46^{B}	**Fe x ST**		
	ST (p = 0.00)		(p = 0.54)	ST (p = 0.76)		(p = 0.67)	ST (p = 0.00)		(p = 0.99)		
Stainless steel pot	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**	(p = 0.00)	**0 H**	**24 H**	(p = 0.07)	**0 H**	**24 H**
Control$^{\#}$	73.18±0.51	75.71±0.63	74.44±1.48^{b}	-2.74±0.04	-2.49±0.06	-2.62±0.14^{a}	5.25±0.31	4.61±0.26	4.93±0.43^{ab}		
NaFeEDTA15	72.78±0.34	74.88±0.54	73.83±1.22^{b}	-2.32±0.14	-2.16±0.03	-2.24±0.13^{b}	4.97±0.19	4.40±0.52	4.68±0.47^{a}	0.64	0.92
NaFeEDTA30	71.74±0.94	74.13±0.52	72.94±1.48^{a}	-2.79±0.05	-2.48±0.14	-2.64±0.20^{a}	5.80±0.28	4.93±0.31	5.36±0.54^{b}	1.54	1.61
Electrolytic iron	71.78±0.21	75.67±0.20	73.72±2.14^{ab}	-2.67±0.13	-2.62±0.06	-2.64±0.10^{a}	5.03±0.46	4.20±0.33	4.62±0.58^{a}	1.42	0.43
Effect of storage time (ST)	72.37±0.82^{A}	75.10±0.80^{B}	**Fe x ST^{1}**	-2.63±0.21^{A}	-2.44±0.19^{B}	**Fe x ST**	5.26±0.44^{A}	4.54±0.42^{B}	**Fe x ST**		
	ST (p = 0.00)		(p = 0.05)	ST (p = 0.00)		(p = 0.11)	ST (p = 0.00)		(p = 0.85)		

^{abc} : per pot type, mean (±SD) values with different superscripts in the same column for effect of iron treatment differ significantly ($p < 0.05$)

^{AB}: per pot type, mean (±SD) values with different superscript for effect of storage time on a colour value differ significantly ($p < 0.05$)

Fe x ST^{1}: Significant interaction between iron (Fe) type and storage time (ST) ($p < 0.05$)

ΔE (colour difference) measured by $[(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$indicates the overall colour relative to control$^{\#}$ porridge

$^{\#}$Porridge fortified with premix excluding iron

4.1.6.8: Sensory scores (appearance and flavour) for iron fortified fresh (0 Hours) and stored (24 Hours) maize porridges prepared in different cooking pots (cast iron, aluminium and stainless steel pots)

For porridges cooked in aluminium pots, the difference -from-control scores for all three iron treatments were not significantly different ($p > 0.05$) from the control in terms of appearance and flavour (Table 4.1.7). The appearance of porridge with NaFeEDTA 15 was significantly more similar to control ($p < 0.05$) compared to porridge with electrolytic iron while no score differences were noted between porridges NaFeEDTA 15 and NaFeEDTA 30 or electrolytic iron and NaFeEDTA 30, respectively in terms of appearance. Stored porridge was significantly more different from control than freshly prepared porridge in terms of appearance while no storage differences were noted for flavour. There was no significant interaction between iron treatment and storage time for the appearance and flavour of porridges ($p > 0.05$).

For porridges cooked in cast iron pots, the difference-from-control score of porridge with electrolytic iron was significantly more different from control while scores for porridges with NaFeEDTA 15 and NaFeEDTA 30 were not different in terms of appearance (Table 4.1.7). For flavour, the difference-from-control score of porridge with NaFeEDTA 30 was more different from control while porridges with NaFeEDTA 15 and electrolytic iron were not. There were no differences in scores between porridges with NaFeEDTA 30 and porridge with electrolytic iron in terms of appearance and flavour. Storage for 24 hours had no significant effect on appearance and flavour of maize porridge ($p > 0.05$). There was no significant interaction between iron treatment and storage time for the appearance and flavour of maize porridges ($p > 0.05$).

For porridges cooked in stainless steel pots, the difference -from-control scores for all three iron treatments were not significantly different from the control in terms of appearance of maize porridges (Table 4.1.7). The difference-from-control score of maize porridge with NaFeEDTA 30 was significantly greater than that of the control while porridges with NaFeEDTA 15 and electrolytic iron were not different in terms of flavour. There were no significant differences between porridges with NaFeEDTA 15 and 30 compared with porridge fortified with electrolytic iron in terms of flavour. Storage for 24 hours had no effect on appearance and flavour of maize porridge ($p > 0.05$). There was no significant interaction between iron treatment and storage time for the appearance and flavour of maize porridges ($p > 0.05$).

Table 4.1.7: Difference - from - control sensory scores* (appearance and flavour) for iron fortified fresh (0 Hours) and stored (24 Hours) maize porridges prepared in different cooking pots (cast iron, aluminium and stainless steel pots)

	Appearance			**Flavour**		
Maize porridge	**Storage Time**		**Effect of Iron (Fe) treatment** (p = 0.02)	**Storage Time**		**Effect of Iron (Fe) treatment** (p = 0.13)
Aluminium pot	**0 Hours**	**24 Hours**		**0 Hours**	**24 Hours**	
Blind control	1.77 ± 0.82^{a}	2.23 ± 0.99^{a}	2.00 ± 0.93^{xy}	2.12 ± 1.07^{a}	2.42 ± 0.95^{a}	2.27 ± 1.01^{x}
NaFeEDTA15	1.93 ± 0.93^{a}	1.81 ± 0.80^{a}	1.87 ± 0.86^{x}	2.50 ± 1.24^{a}	2.54 ± 0.99^{a}	2.52 ± 1.11^{x}
NaFeEDTA 30	1.85 ± 0.92^{a}	2.19 ± 0.94^{a}	2.02 ± 0.94^{xy}	2.92 ± 1.32^{a}	2.54 ± 0.95^{a}	2.73 ± 1.16^{x}
Electrolytic iron	2.16 ± 0.94^{a}	2.65 ± 1.02^{a}	2.41 ± 1.00^{y}	2.19 ± 0.98^{a}	2.50 ± 0.99^{a}	2.35 ± 0.99^{x}
Effect of storage time (ST)	1.93 ± 0.90^{A}	2.22 ± 0.98^{B}	**Fe x ST**	2.44 ± 1.19^{A}	2.50 ± 0.96^{A}	**Fe x ST**
	ST (p = 0.02)		(p = 0.31)	ST (p = 0.65)		(p = 0.31)
Cast iron pot	**0 Hours**	**24 Hours**	**Effect of Fe** (p = 0.03)	**0 Hours**	**24 Hours**	**Effect of Fe** (p = 0.03)
Blind control	1.68 ± 1.00^{a}	1.79 ± 0.89^{a}	1.73 ± 0.94^{x}	2.19 ± 0.96^{a}	2.15 ± 0.95^{a}	2.16 ± 0.95^{x}
NaFeEDTA 15	1.85 ± 0.99^{a}	2.04 ± 0.85^{a}	1.95 ± 0.92^{xy}	2.59 ± 1.31^{a}	2.52 ± 1.09^{a}	2.55 ± 1.19^{xy}
NaFeEDTA 30	2.04 ± 0.98^{a}	2.00 ± 1.07^{a}	2.02 ± 1.02^{xy}	3.07 ± 1.11^{a}	2.44 ± 1.12^{a}	2.76 ± 1.15^{y}
Electrolytic iron	2.07 ± 1.00^{a}	2.48 ± 1.01^{a}	2.28 ± 1.02^{y}	2.33 ± 1.00^{a}	2.41 ± 0.84^{a}	2.37 ± 0.92^{xy}
Effect of storage time (ST)	1.91 ± 0.99^{A}	2.08 ± 0.98^{A}	**Fe x ST**	2.54 ± 1.14^{A}	2.38 ± 1.00^{A}	**Fe x ST**
	ST (p = 0.21)		(p = 0.69)	ST (p = 0.25)		(p = 0.31)
Stainless steel pot	**0 Hours**	**24 Hours**	**Effect of Fe** (p = 0.28)	**0 Hours**	**24 Hours**	**Effect of Fe** (p = 0.01)
Blind control	1.96 ± 1.14^{a}	1.82 ± 0.94^{a}	1.89 ± 1.04^{x}	2.36 ± 1.16^{a}	2.04 ± 1.20^{a}	2.18 ± 1.18^{x}
NaFeEDTA 15	2.11 ± 0.99^{a}	2.11 ± 0.92^{a}	2.11 ± 0.95^{x}	2.57 ± 1.00^{a}	2.61 ± 0.96^{a}	2.56 ± 0.97^{xy}
NaFeEDTA 30	2.04 ± 1.14^{a}	2.14 ± 0.93^{a}	2.09 ± 1.03^{x}	3.11 ± 1.28^{a}	2.93 ± 0.94^{a}	3.01 ± 1.11^{y}
Electrolytic iron	2.14 ± 1.11^{a}	2.39 ± 0.83^{a}	2.27 ± 0.98^{x}	2.46 ± 1.00^{a}	2.71 ± 1.01^{a}	2.58 ± 1.01^{xy}
Effect of storage time (ST)	2.06 ± 1.08^{A}	2.12 ± 0.92^{A}	**Fe x ST**	2.61 ± 1.14^{A}	2.56 ± 1.07^{A}	**Fe x ST**
	ST (p = 0.69)		(p = 0.77)	ST (p = 0.69)		(p = 0.50)

*1 (same as control) to 5 (extremely different from control)

^{xy}: per pot type, mean (±SD) values with different superscript for effect of iron treatment differ significantly ($p < 0.05$)

^{AB}: perpot type, mean (±SD) values with different superscript for effect of storage time on appearance and flavour differ significantly ($p < 0.05$)

^{ab}: per pot type, mean (±SD) values with different superscript for effect of interaction of treatment and storage time differ significantly ($p < 0.05$)

Fe x ST: Interaction between iron (Fe) type and storage time (ST)

4.1.7 Discussion of results

The fat content of the unfortified maize meal did agree with the South African regulation for special maize meal (79 % extraction rate) (Regulation for dry-milled maize products - dry - milled maize products - Johnson *et al.*, 2004) which prescribe a maximum oil content of 3.0 % (dry basis) but had lower ash content as compared to the prescribed value of 0.85 %. Oikeh, *et al.*, (2003) observed that the mineral content of maize kernels varies due to genetic and environmental factors. This could possibly explain the lower ash (0.60 %) content observed in this study. The maize meal was obtained from adequately dried grains with moisture content of 12.70 %. The analysis of iron content detects not only the iron added by fortification, but also intrinsic iron in the cereal flours (Van den Wijngaart and Codling, 2013). A higher intrinsic iron content (7.65 mg iron/kg maize meal) observed in this study explains why in some cases the final iron content of the maize meals is higher than the expected dosage of added iron in the fortified maize meal.

Electrolytic iron maize meal is the only sample that showed significantly lower L* and a* values when compared to the control. This lower L* value could be attributed to elemental iron powders, (e.g. electrolytic iron) being black powders and these have been reported to cause some slight darkening when added to maize flour (Johnson *et al*., 2004). NaFeEDTA 15 and NaFeEDTA 30 maize meals had significantly higher b* (more yellow) values when compared to maize meal with electrolytic iron. This could be because NaFeEDTA is pale yellow in colour (Bothwell and MacPhail, 2004). Similar findings were observed by Richins *et al.*, 2008 who observed that corn flour tortillas fortified with NaFeEDTA at 16.98 mg iron /kg had a significantly higher b* value (42.76) than corn flour tortillas fortified with electrolytic iron at 80 mg iron/kg with b* value (24.92).

According to the spot iron test, there was an increase in a* values (more red) with increase in iron concentration in maize meal. An increase in the number of red spots was observed with increase in iron concentration in maize meal. Electrolytic iron showed up as tiny highly visible dots while NaFeEDTA showed up as larger spots. The differences in the type of iron fortificant compound could have contributed to size and shape of the red spots formed. The number and distribution of red spots reflects roughly the amount and distribution of the iron in iron fortified cereals (Johnson *et al.*, 2004). The development of red spots has been attributed to the reaction between ferric iron (Fe^{3+}) from the fortificant premix and possibly intrinsic iron with the thiocyanate ion (SCN^-) from potassium thiocyanate (KSCN) reagent

(Johnson *et al.*, 2004). A significant decrease in L* values with increase in iron concentration was observed. This could be attributed to the loss of the lightness component of the maize meals due to the development of the red and yellow colour components. Commercially fortified maize meal and that fortified with electrolytic iron or NaFeEDTA 15 and 30 had significantly higher a* values (more red). This could be attributed to the reaction between ferric iron (Fe^{3+}) and thiocyanate ion (SCN^-). Commercially fortified maize meal and that fortified with electrolytic iron had significantly higher b* values (more yellow) compared with maize meal with NaFeEDTA 15 and 30. The significantly higher b* colour values were due to the development of the yellow colour that could have been due to the addition of hydrogen peroxide (H_2O_2) used to oxidise ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron. This addition is only required in maize meals fortified with electrolytic iron and not NaFeEDTA.

Cooking maize porridges in aluminum pots did not result in significant differences in the L*, a* and b* values of the NaFeEDTA 15 and 30, compared to electrolytic iron fortified porridge. However, storage for 24 hours resulted in higher L* and lower a* values. Cooking maize porridges in cast iron pots resulted in higher b* value of the NaFeEDTA 15 compared to electrolytic iron fortified porridge. NaFeEDTA 30 had higher L* and b* values compared to the electrolytic iron fortified porridge. Storage for 24 hours resulted in higher L*, and lower b* values. Cooking maize porridges in stainless steel pots resulted in higher a* value of the NaFeEDTA 15 compared to electrolytic iron fortified porridge. NaFeEDTA 30 had higher b* value compared to electrolytic iron fortified porridge. Storage for 24 hours resulted in higher L* and a* but lower b* values. The differences in colour of the NaFeEDTA 30 or NaFeEDTA 15 porridges when compared to electrolytic iron fortified porridge that was observed in cast iron, stainless steel pots but not in aluminium pot could be attributed to different iron treatments. Although electrolytic iron may have reduced bioavailability compared to NaFeEDTA, it had the least effect on colour of food vehicles (Richins *et al.*, 2008; Kiskini *et al.*, 2010). Electrolytic iron is water insoluble and also poorly soluble in dilute acid and this contributes to its stability when added to cereal products like maize meal (Hurrell, 2002a). Off-colour formation in iron fortified cereal flours has been attributed to the reaction between iron ions and polyphenols found in the cereals (Mellican *et al.*, 2003; Hurrell, 1997). Storage of porridge for 24 hours resulted in higher L* and lower a* (aluminium pot), higher L* and lower b* (cast iron pot) and higher L*, higher a* and lower b* (stainless steel pot). These findings suggest that the stainless steel cookware led to more colour changes of porridge when stored for 24 hours. However these findings do not agree with the hypothesis of the study as it was expected that more colour changes would be observed in the porridges that were prepared in cast iron or aluminium pot. With time, the contact time between polyphenols (ferulic and p-coumaric acid) found in maize meal (Pozo-Insfran *et al.*, 2006) and iron ions probably contributed to the colour changes. The difference in the type of cooking pots might also have contributed to these colour differences. These pots differ in their thermal conductivity and heat transfer (Tilley, 2004), and levels and type of ions leaching into the food during cooking (Verissimo *et al.*, 2006; Bi, 1995; Kuligowski and Halperin, 1992). The practical implications are that from the L* a* b* results it is not clear whether or not these colour differences will be noticed by the human eye.

The difference-from-control scores for all three iron treatments were not significantly different from that of the control (porridge fortified with premix excluding iron) in terms of appearance and flavour of maize porridges cooked in aluminium pots as judged by the analytical sensory panel. A similar pattern was observed for maize porridge prepared in stainless steel pots but not in cast iron pots in terms of appearance. The difference in colour (ΔE) values compared to control porridge for fresh and stored porridges ranged from 0.47 to 1.54 and 0.40 to 1.61 respectively. This ΔE indicated that a possible noticeable visual change in colour due to iron treatment could be perceived by the analytical sensory panel. However, Francis and Clydesdale (1975) indicated that only $\Delta E > 2$ would correspond to noticeable differences in the visual perception of many food products. In this study none of the ΔE values of the either fresh or stored porridges was greater than two ($\Delta E > 2$). Although all the ΔE values were less than two ($\Delta E > 2$) the sensory panel did observe visual colour differences between control porridge and that fortified with electrolytic iron prepared in cast iron pot. However, porridges fortified with NaFeEDTA at either 15 or 30 mg iron/kg maize meal were similar to control porridge in terms of appearance. Electrolytic iron is however water insoluble and has been reported to have low reactivity with food matrices leading to few colour changes in iron fortified foods (Hurrell *et al.*, 2002). Similar findings to the one observed in this study have been reported by Randall *et al.*, (2012) who observed in Tanzania and Kenya that ugali (stiff maize porridge) prepared from maize meal fortified with NaFeEDTA at 15 mg iron/kg had slightly different colour from unfortified sample but had normal taste as assessed by trained panellists. Maize meal ugali prepared from maize meal fortified with NaFeEDTA at 20 mg iron/kg was reported as having normal colour, taste, texture and aroma. Van den Wijngaart and Codling, (2013) reported that flour fortified with NaFeEDTA at 20 mg iron/kg produced wet noodles with a similar colour to wet noodles produced from unfortified flour. Dissimilar findings to the one observed in this study have been reported by Bovell-Benjamin *et al.*, (1999), who observed that maize porridge prepared from whole maize meal fortified with NaFeEDTA at 30 and 60 mg iron/kg had the dullest colour when compared to the control porridge (prepared from unfortified maize meal).

Contradictory findings to that observed in this study about effects of iron in the form of NaFeEDTA were also reported by Moretti *et al.,* (2005), who observed that extruded rice grains fortified with NaFeEDTA at 5 mg iron/100 g rice (50 mg iron/kg) did show some significant colour changes when compared to unfortified samples. Only stored maize porridge prepared in aluminium pots was more different from control than freshly prepared porridge in terms of appearance. This could be attributed to more contact time between the iron ions (Fe^{3+}/Fe^{2+}) and polyphenols (ferulic acid and p-coumaric acid) found in maize meal leading to more intense colour changes. The main polyphenols found in maize meal are ferulic and p-coumaric acid (Pozo-Insfran, *et al.,* 2006). Hurrell (1997) suggested that polyphenols may be involved in the off-colour development of iron fortified foods. A study done in model solutions and in foods rich in polyphenols confirmed this suggestion by showing that ortho-hydroxy groups as found in gallic acid (e.g. chocolate), catechin (found in green tea) and chlorogenic acid (in coffee) cause off-colour developments with iron (Mellican *et al.,* 2003). Off-colour formation in iron fortified cereal flours has been attributed to the reaction between iron ions and polyphenols found in the cereals. Iron is a transition metal; it participates in oxidation/reduction reactions: $Fe^{2+} \longrightarrow Fe^{3+} + e^-$ (Mellican *et al.,* 2003; Hurrell, 1997).

Maize porridge with NaFeEDTA 30 mg iron/kg prepared in cast iron and stainless steel pots had a difference-from-control score which was significantly greater than that of the control in terms of flavour as judged by the sensory panel. The flavour difference could be due to iron catalysed lipid oxidation. Linoleic acid found primarily in plant oils is the main polyunsaturated fatty acid (PUFA) in maize meal and it is susceptible to lipid oxidation (Bovell-Benjamin, *et al.,* 1999). Maize porridges with electrolytic iron prepared in cast iron pot were significantly different from the control in terms of appearance. This could be attributed to the oxidation/reduction reactions between the iron ions and polyphenols (ferulic acid and coumaric acid) found in maize porridge which leads to the formation of coloured compounds and thereby leading to colour changes in maize porridge.

In general, cast iron pots did show significant differences due to iron treatment in both appearance and flavour of porridge while stainless steel pots did show significant differences due to iron treatment only in the flavour of porridge and aluminium pots showed significant differences due to iron treatment only in appearance of porridge. These differences in results for the cooking pots could be attributed to the differences in thermal conductivity and heat transfer of different cooking material (Tilley, 2004) and possible leaching of iron ions (Fe^{2+}/Fe^{3+}) from cast iron and stainless steel pots and aluminium (Al^{3+}) ions from aluminium pots at different rates from different cooking materials during the cooking process (Verissimo *et al.*, 2006; Bi, 1995; Kuligowski and Halperin, 1992). These leaching iron ions (Fe^{2+}/Fe^{3+}) may possibly lead to additional iron ions that contribute to the oxidation/reduction reactions that lead to colour formation the maize meal porridge. Aluminium (Al^{3+}) is more reactive than Fe^{3+} hence it will displace the iron ions in any chemical compound (Holman and Stone, 2001). These displaced iron ions may possibly contribute to more iron ions in the food matrix leading to more oxidation/reduction reactions and thus more colour changes.

Significant effect of storage time was observed only in appearance of maize porridge prepared in aluminium pot and not in cast iron and stainless steel. There was no significant effect of storage in the flavour of porridge prepared in all the three cooking materials (aluminium, cast iron and stainless steel pots). There was no significant interaction effect of iron treatment and storage for appearance and flavour of maize porridges in all three types of cooking material (aluminium, cast iron and stainless steel pots).

4.1.7 Conclusions

Overall, it appears that a change from fortifying maize meal with electrolytic iron at 35 mg iron/kg (current multi-micronutrient fortificant premix) to NaFeEDTA at 15 mg iron/kg (proposed premix level) will not lead to changes in appearance and flavour of maize porridge. Even an excess amount of NaFeEDTA at 30 mg iron/kg will not be detected as a change. The more bioavailable form of iron (NaFeEDTA) could be a viable option to reduce prevalence of iron deficiency in developing countries. Although there were no significant differences between porridge fortified with the current multi-micronutrient fortificant premix and that fortified with the multi-micronutrient fortificant premix with NaFeEDTA at 15 mg iron/kg or 30 mg iron/kg, it is recommended to verify the acceptability of the porridges using regular consumers of the product.

4.2 Consumer sensory evaluation of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron and stainless steel pots

Abstract

Iron deficiency, and specifically iron deficiency anaemia, remains one of the most severe and important nutritional deficiencies in the world today. Because of the widespread nature of iron deficiency, iron is considered a basic component in most food-fortification programmes. However, the iron compounds used may lead to undesirable sensory changes, especially to the colour and flavour of the foods being fortified. This study evaluated the effects of sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) and electrolytic iron on consumer acceptance of the colour and flavour (taste) of fortified special maize meal prepared as stiff porridge using two different types of cooking vessels; stainless steel and cast iron pots. Maize meal was fortified with the standard multi-micronutrient premix containing vitamins, zinc and calcium with either NaFeEDTA at 30 mg iron/kg maize meal (double the dose necessary) or electrolytic iron at 35 mg iron/kg maize meal. Porridges fortified with NaFeEDTA or electrolytic iron were prepared in cast iron and stainless steel pots for acceptance testing by consumers. A total of eighty (80) consumers (54 females and 26 males) between 18 and 74 years, who were regular consumers of maize porridge (3 or more times a week), were used in the study. The type of iron did not have a significant effect on acceptability of colour and taste of porridges cooked in stainless steel pots. When prepared in cast iron pots, the type of iron did have a significant effect ($p < 0.05$) on acceptability of taste, where porridge fortified with electrolytic iron was liked more than those with NaFeEDTA at 30 mg iron/kg maize meal. The type of iron did not have a significant effect on acceptability of colour of porridges prepared in cast iron pots. However, overall, the porridges prepared in stainless steel cookware were significantly liked more ($p < 0.05$) compared to those prepared in cast iron cookware. NaFeEDTA at double the dose anticipated might lead to acceptability (taste) problems if porridge is prepared in cast iron cookware.

4.2.1 Introduction

The research aim for this part of the study was to determine the level of acceptability of porridges prepared from special maize meal fortified with a multi-micronutrient premix containing NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal. Although there were no significant differences ($p > 0.05$) in the sensory properties between porridge fortified with the multi-micronutrient premix containing electrolytic iron at 35 mg iron/kg maize meal and those fortified with the multi-micronutrient premix with NaFeEDTA at 15 mg iron/kg maize meal or 30 mg iron/kg maize meal as judged previously by an analytical sensory panel (4.1.4.3), it was recommended to verify acceptability of the porridges using a consumer panel of regular maize meal users.

The porridges were prepared in stainless steel and cast iron pots and evaluated by regular consumers of maize porridge. Electrolytic iron at 35 mg iron/kg maize meal forms part of the current legislated multi-micronutrient premix for maize meal in South Africa (Department of Health, South Africa, 2003) while NaFeEDTA at 15 mg iron/kg maize meal is the proposed new form of iron compound and dosage level to be used as part of the multi-micronutrient premix for maize meal in South Africa (Personal communication; M. Hoop, Department of Health, South Africa, 2014). NaFeEDTA at 30 mg iron/kg maize meal, which is double the recommended dosage, was considered here.

Sensory evaluation by panellists trained to detect differences of maize porridges fortified with multi-micronutrient premix having zero iron added, electrolytic iron at 35 mg iron/kg added or NaFeEDTA at 15 or 30 mg iron/kg added and prepared using different types of cooking pots was reported earlier in chapter 4 (section 4.1.3). The appearance of maize porridges prepared in cast iron cooking pots, with electrolytic iron at 35 mg iron/kg maize meal was significantly different ($p < 0.05$) from the zero iron added control porridge. The flavour of maize porridge with NaFeEDTA at 30 mg iron/kg maize meal was also significantly different ($p < 0.05$) from the control. When preparing maize porridge with NaFeEDTA at 30 mg iron/kg in stainless steel pots, the flavour was significantly different ($p < 0.05$) from the control. The objective of this part of the research was to determine whether the appearance and/or flavour differences related to iron treatment and/or choice of pot noted by the analytical panel would have an effect on the acceptability of maize porridges by regular maize porridge consumers.

4.2.2 Consumers

A total of eighty (80) consumers (54 females and 26 males) between 18 and 74 years, who are regular consumers of maize porridge (3 or mores time a week) were recruited by an independent recruitment agent in Mamelodi, Tshwane metropole, South Africa to participate in the study on Monday 15 June 2015. The sensory evaluation exercise was held at the University of Pretoria, Mamelodi campus. Consumers (10 at a time) were invited to participate in a 30 minute session where they evaluated four samples of maize porridge. Each consumer was assigned to an interviewer who explained the evaluation procedure and led the evaluation interview style using, where necessary, one of the local non-English languages. The consumers completed and signed a consent form after taking part in the study. The consumers received monetary compensation in the form of R 50 store voucher for their time.

4.2.3 Maize porridge preparation

The same method of maize porridge preparation as used in sensory evaluation (difference-from-control) (section 4.1.3.1) was used. Stiff maize porridge prepared as described in section 4.1.3.1 was prepared in two identical cast iron pots (3 liters in volume) and two identical stainless steel pots (3.2 liters in volume). Maize porridge containing a multi-micronutrient premix as described in section 4.2.1 which includes NaFeEDTA at 30 mg iron/kg maize meal and porridge with electrolytic iron at 35 mg iron/kg maize meal was prepared in the cast iron cooking pots and stainless steel pots. The pots were placed on hot trays during serving to keep the porridges warm (± 52 ^{o}C). A total of 40 porridge samples, each weighing 30 - 35 g were served from each pot. Two cooking cycles were used to serve a total of 80 consumers.

4.2.4 Sample coding and evaluation

At the commencement of the evaluation process, the interviewers explained the purpose of the study to the consumers. After each consumer was clear with their role in the exercise, they were required to wash their hands before commencing with the taste test. The washing of hands took place at the entrance as consumers came in for their maize porridge tasting session. The interviewer gave a comprehensive explanation of both the consent form as well as the evaluation form/questionnaire (Appendix A). Note that the results for some of the questions (Question 12; Question 18; Question 20; Question 21) asked in the questionnaire were not reported as part of this dissertation. The validity and reliability of the questionnaire was not formally evaluated. Due to the limited level of literacy expected from some of the consumers and also as a way to allow the consumer to focus on the food and not on a complex questionnaire, each consumer was assigned to an interviewer who read the evaluation form step by step before the consumer tasted the porridge and in some cases, a translator was needed in order to make sure that the evaluation form was properly and accurately understood.

The four cooked porridge samples (30 - 35 g each), in 40 ml plastic containers with plastic lids to prevent loss of volatiles were served simultaneously. The samples were coded with randomly selected 3-digit codes and presented to the consumers following a Williams design (Joeng *et al.*, 2013).

Participants were provided with damp disposable unscented towels with which to wipe their hands between tasting of samples since they had to press the porridge between their fingers in order to evaluate the cooked porridges. Traditionally porridge is eaten using hands in South Africa as opposed to the use of cutlery in the western culture. A five point hedonic scale (face scale) (Figure 4.2.1) was used to measure the overall acceptance of colour and taste of each sample. A score sheet shown in Appendix A was used to capture the data. The meanings of the faces on the evaluation form were explained to the consumers by the interviewers. The faces on the scale were supported by word phrases: “Dislike very much”, “Dislike a little”, "Not sure", “Like a little” and “Like very much”. According to Stone and Sidel, (1992), face scales are suitable for those with limited reading and/or comprehension skills. We expected some of the participants in the study to be illiterate or semi-illiterate.

Individual porridge samples were prepared and served in view of consumers to ensure transparency. Consumers were also prompted to provide comments on the reasons why they liked or disliked the different porridges (Appendix B). Coetzee, (1997) stated that traditional sensory evaluation methods should be modified to suit the level of education and cultural fears of low literate respondents. The participants rinsed their mouths with water at room temperature between tasting of each individual cooked porridge samples so as to neutralise their palates.

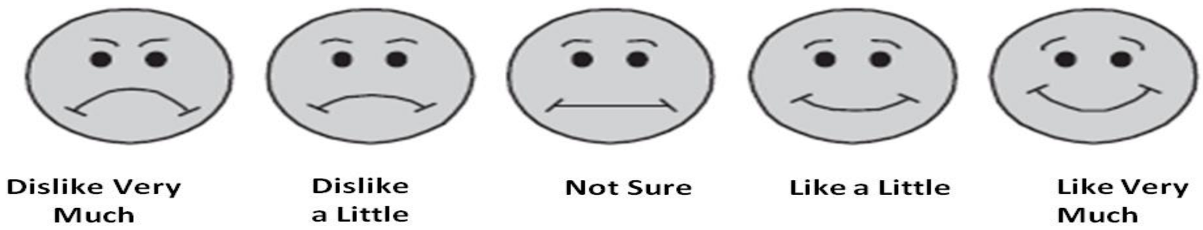


Figure 4.2.1: Five point hedonic scale (face scale)

4.2.5 Statistical analysis

The data obtained was coded as follows: 1 = “Dislike very much”, 2 = “Dislike a little”, 3 = "Not sure" 4 = “Like a little”and 5 = “Like very much”and then entered on an Excel spread sheet for analysis using Statistical Package for the Social Sciences (SPSS) version 22. Two-way analysis of variance (ANOVA) with porridge and consumers as main effects was used to determine the acceptability of colour and taste of the porridge. Statistical analyses were performed using SPSS version 22 (SPSS, Chicago, USA). Where significant effects were noted, Tukey Honestly Significant Difference HSD test at $p < 0.05$ was used to compare means.

4.2.6 Results

4.2.6.1 Consumer acceptability of colour and taste of maize porridges

The type of iron did not have a significant effect ($p > 0.05$) on acceptability of colour and taste of porridges cooked in stainless steel pots (Table 4.2.1). The type of iron did however have a significant effect on acceptability of taste but not colour of porridges prepared in cast iron pots. Additionally, porridge fortified with electrolytic iron that was prepared in stainless steel pot was better liked compared to porridge fortified with either electrolytic iron or NaFeEDTA prepared in cast iron pot in terms of colour. Porridge with electrolytic iron that was prepared in stainless steel pot was better liked compared to porridge with NaFeEDTA prepared in cast iron pot in terms of taste.

4.2.6.2 Frequency of maize meal consumption; consumer preferred maize meal brands; knowledge and thoughts regarding maize meal iron fortification

The average days in a week of consumption of maize meal porridge by consumers participating in the study are displayed in Table 4.2.2. Consumer’s knowledge and thoughts regarding addition of iron in maize meal is also displayed. Consumers preferred maize meal brands are also shown.

Table 4.2.1: Consumer acceptability (colour and taste) of maize porridges from maize meal fortified with either NaFeEDTA at 30 mg iron/kg or electrolytic iron at 35 mg iron/kg prepared in either cast iron or stainless steel pots

Maize porridge and pot type	Colour (p = 0.01)	Taste (p = 0.02)
Electrolytic iron cast iron	3.05 ± 1.32^{a}	3.71 ± 1.43^{b}
NaFeEDTA cast iron	3.09 ± 1.30^{ab}	3.16 ± 1.56^{a}
NaFeEDTA stainless steel	3.52 ± 1.16^{bc}	3.64 ± 1.24^{ab}
Electrolytic iron stainless steel	3.65 ± 1.10^{c}	3.91 ± 1.12^{b}

^{abc} = mean (±SD) values with different superscripts in the same column, differ significantly ($p < 0.05$)
Acceptability was measured on a five point hedonic scale (face scales) from 1= Dislike very much to 5 = like very much

Table 4.2.2: Frequency of maize meal consumption; preferred maize meal brands; knowledge and thoughts regarding maize meal iron fortification of the consumers that participated in this study (n = 80 consumers)

Distribution on consumption frequency (% consumers) of maize porridge per week							Did you know that iron (Fe), a micronutrient, has to be added to maize meal in South Africa		Do you think it is a GOOD or BAD idea to add iron (Fe) to maize meal?			Maize meal brands consumed/preferred by the consumers (%)	
1 day	2 days	3 days	4 days	5 days	6 days	7 days						Brands	(%)
2.50%	10.00%	20.00%	18.75%	8.75%	13.75%	26.25%	32.50 % of the consumers knew	67.50% of the consumers did not know	92.50 % of the consumers said it was good idea	5. 00 % of the consumers said it was a bad idea	2.50 % of the consumers were not sure	Super Sun	48.75 %
												White Star	13.75 %
												Ace	12.50 %
												Blue Bird	7.50 %
												Lion	6.25 %
												Iwisa	6.25 %
												Cup Final	2.50 %
												Shaya	1.25 %
												Tafelberg	1.25 %

4.2.7 Discussion of results

Maize porridge fortified with NaFeEDTA at 30 mg iron/kg and maize porridge fortified with electrolytic iron were at statistical parity in terms of acceptability of both colour and taste when using stainless steel cookware. Cooking porridge in cast iron cookware resulted in electrolytic iron fortified porridge being scored significantly higher than porridge fortified with NaFeEDTA 30 mg iron/kg in terms of taste. This was not expected since no difference was found when the analytical panel compared the same porridges. The higher taste score of electrolytic iron fortified porridge could be due to a number of factors.

Consumer sensory evaluation was done three months after analytical sensory evaluation had been conducted. Although the maize meal was stored inside air tight containers and refrigerated (3 - 5 °C), the three months storage could have allowed the iron compounds to interact with polyunsaturated fatty acids found in maize meal, particularly linoleic acid. This interaction could have possibly lead to flavour changes in the maize porridge. Bovell-Benjamin *et al.*, (1999) reported that iron fortification of maize meal does contribute to lipid oxidation of linoleic acid leading to the development of hexanal, a major off-flavour compound in iron fortified maize meal porridge. Electrolytic iron is water insoluble and very stable (Hurrell, 2002a) while NaFeEDTA is slightly water soluble (Bothwell and MacPhail, 2004). The reactivity of iron compounds with food components increases with an increase in their water solubility (Hurrell, 1997). This suggests that NaFeEDTA, being more water soluble than electrolytic iron could have contributed more to reactions that lead to flavour changes with increased storage time of the maize meal. Although electrolytic iron may have reduced bioavailability compared to NaFeEDTA, it has the least effect on the flavour of food vehicles (Hurrell, 1997).

This contradictory finding between the analytical and consumer panel could also be attributed to the power of the test methods used. The power of the test (β) is defined as the probability of finding a difference if one actually exists (Lawless and Heymann, 1998). The power of the test is dependent on the magnitude of the difference between the samples, the statistical significance level or p-value applied, and the number of panellists performing the test (Lawless and Heymann, 1998). Twenty eight panelists were used for analytical sensory evaluation while eighty (80) panellists were used for consumer sensory evaluation.

In general, porridge prepared in stainless steel pots were more acceptable compared to porridge prepared in cast iron pots. Cast iron cookware, which is typically mostly iron

(Kuligowski and Halperin, 1992) has been reported to leach significant amounts of Fe ions (Fe^{2+}/Fe^{3+}) during the cooking process (Verissimo, *et al.*, 2006; Bi, 1995; Kuligowski and Halperin, 1992). The leached iron ions (Fe^{2+}/Fe^{3+}) could have possibly lead to additional iron ions that contributed to the lipid oxidation of linoleic acid leading to more flavour changes of the porridge. These additional iron ions (Fe^{2+}/Fe^{3+}) could also have contributed to additional oxidation/reduction reactions between the iron ions and polyphenols (ferulic acid and coumaric acid) found in maize porridge thereby leading to the formation of more coloured compounds and thus more colour changes in the maize porridge.

A total of 87.5 % consumers were regular consumers of stiff maize porridge because they consumed the porridge for an average of at least three (3) or more times in a week. Choosing regular consumers of the maize porridge ensured that the consumers had an existing frame of reference and thereby were able compare the product with similar products that they had tried. It also ensured that the consumers possessed reasonable expectations on the product (Lawless and Heymann, 2010). A significant portion (92.50 %) of the consumers felt it was a good idea to add iron into the maize meal. Super Sun (48.75 %), White Star (13.75 %) and Ace (12.50 %) were the most popular maize meal brands among the consumers.

4.2.8 Conclusions

The colour of maize porridge fortified with either NaFeEDTA at 30 mg iron/kg maize meal or 35 mg electrolytic iron/kg maize meal will not lead to acceptability problems of porridge prepared in stainless steel and cast iron cookware. However, the concentration of NaFeEDTA as used in this study namely 30 mg iron/kg maize meal i.e. twice more than what is considered by the national Department of Health might lead to acceptability (taste) problems if porridge is prepared in cast iron cookware. Strict control and monitoring of the addition of the fortificant and the prevention of overdosing is therefore recommended. Once the replacement of electrolytic iron with NaFeEDTA is mandatory, it is recommended that further house-hold testing involving regular consumers of maize meal using their own cookware and porridge preparation equipment and methods be done.

In the long run, fortification of maize meal porridge with a more bioavailable source of iron (NaFeEDTA) may reduce the prevalence of iron deficiency in South Africa.

CHAPTER 5: GENERAL DISCUSSION

This chapter comprises two main sections. The first section critically discusses the development of the experimental design, the challenges with procurement of some of the materials and methodologies used during this study, including principles, strengths and weaknesses. The second phase will critically evaluate the colour of iron fortified maize meal and the sensory properties of stiff porridges prepared from the maize meal.

5.1 Critical evaluation of experimental design and methodologies used

5.1.1 Physico-Chemical analyses and instrumental methods

Unfortified special maize meal (79 % extraction rate) used in this study was obtained from Premier Milling, Pretoria, South Africa. Special maize meal contributes a significant part of the market share (36 %) which is equivalent to that of super maize meal (63 % extraction rate) (Johnson *et al.*, 2004). Multi-micronutrient fortificant premixes were prepared by DSM Nutritional Products South Africa (Pty) Ltd. (Isando, South Africa). Three fortificant premixes were supplied containing electrolytic iron or NaFeEDTA and a control premix with no iron added. The mixing of the fortificant premix and maize meal was done at the Southern African Grain Laboratory (SAGL) following internal accredited standard procedures.

Three types of commonly used cooking vessels (aluminium, cast iron and stainless steel pots) were used in the preparation of maize meal porridge in the study. Four aluminium and four stainless steel pots were already available in the Department of Food Science pilot plant while obtaining four cast iron pots took a lot of time (3 months) as the cookware was not available in most shops. It was also difficult and finally impossible to find pots made of the three different materials of the same volume. The delay in obtaining the cast iron cookware in addition to the delay in obtaining the fortificant premixes from DSM presented some of the practical challenges faced in conducting the scientific research project.

A typical South African stiff porridge-making process (Kuyper *et al.,* 2000) with modifications was used for the preparation of the stiff maize porridges. Stiff maize meal porridge is a staple food in South Africa and is associated with low income earning consumers who are also the most vulnerable to micronutrient malnutrition (National Food Consumption Survey, 2000). The stiff maize porridge preparation phase was a critical part of the scientific research. A total of four stiff maize porridges from differently iron treated maize meals were prepared simultaneously following a prescribed cooking recipe which required a total of four cooking assistants per cooking cycle. To ensure that the cooking assistants were able to follow the cooking recipe precisely, a one hour training session was conducted an hour before each cooking cycle. The recipe was straight forward and easy to follow as indicated by most of the cooking assistants that were involved in the cooking process. This training session ensured that the procedures were standardised and thereby minimising any potential differences in the end porridge due to variability among the cooking assistants. Some of the cooking assistants were regular consumers and familiar with the preparation of stiff maize porridge and this made the training process less challenging.

Qualitative analysis of iron from fortification (presence and distribution) was determined using the spot iron test AACC Method 40 - 40 (AACC, 2000). With this method, ferric iron (Fe^{3+}) from NaFeEDTA in an acidic medium reacts with a solution of potassium thiocyanate (KSCN) to form an insoluble red pigment. Other types of iron, such as ferrous iron and elemental iron can also react in a similar manner once they are oxidised to ferric form using hydrogen peroxide (H_2O_2) (Johnson *et al.*, 2004). In this study, the unfortified maize meal sample did show a reddish colouration, but not as well defined as the obvious red spots in fortified and commercially fortified samples. The number and density of red spots reflects roughly the amount and distribution of iron in the sample. Control maize meal (e.g. in this case commercially fortified) was used to make comparative assessment of the range of iron content in the fortified samples (Nichols, Aburton, Masad, Wirth, Sullivan and Serdula, 2012). Although there is no rule for the size of the spots, their appearance might vary from small, well defined, to large spots tending to diffuse as the iron solubilises. This might be due to the source and quality of iron used to fortify the maize meal (Johnson *et al.,* 2004). The advantage of this method is its simplicity. However, the disadvantage is that it is only qualitative and not quantitative.

Colour (L* a* b*) of uncooked maize meal and maize porridge was determined using a Minolta Chroma Meter CR-400/410 instrument, (Konica Minolta Sensing, Japan). The instrument expresses colour in L*, which denotes lightness, a* denotes the red/green value and b* denotes the yellow/blue value. The most visible effect of iron compounds on the fortified food has been reported to be the change in colour of the food. When using highly bioavailable iron, foods and beverages can dramatically change colour (Mellican *et al.,* 2003). The colour of food can strongly influence the perception of the taste of that food (O’Donnell, 1997). Many consumers use the colour of a food product to indicate the quality of the product (Clydesdale, 1998). Using instrumental colour measurement, an objective approach, should offer quantitative measures of colour quality which is more reliable to the subjective measurements done sensorially (Pathare, Opara and Al-Said, 2013). Objective colour measurements are used to relate colour measurements to the tristimulus sensory perception of colour by the human eye (HunterLab, 1995). Therefore, the International Committee on Illumination (CIE) has adopted colour measurements instrumentally by the use of X, Y, Z or L* a* b* (CIELAB) values as numerical representative of the three sensory colours by the human eye (McGuire, 1992; Pathare *et al.*, 2013). In the CIELAB colour space, the lightness coefficient, L* ranges from black = 0 to white = 100 (McGuire, 1992); a*: negative values indicate green, positive values indicate red; b*: negative values indicate blue, positive values indicate yellow (Richins *et al.*, 2008). In general, instrumental colour measurements provide relatively precise colour evaluation as it avoids the effect of differences in colour perception by humans (Voss, 1992). Conventional colour meters such as the CR-210 Minolta chromameter have been used objectively to determine colour differences in iron fortified extruded rice grains (Moretti *et al.*, 2005); iron fortified wheat bread (Kiskini *et al.*, 2010) and in iron fortified corn flour tortillas (Richins, *et al.*, 2008) by using the L* a* b* colour scale (Voss, 1992). Apart from differences in instrumentation, colour measurements are often reported based on different colour indices even for the same product, making it difficult to compare results in the literature. There is need for standardisation to improve the traceability and transferability of measurements (Pathare *et al.*, 2013). The disadvantage of the instrumental colour analysis is that it does not tell whether or not the colour of the food is acceptable or not.

In order to evaluate the total colour changes between the maize porridge samples, colour difference (expressed as ΔE) was analysed in this study. To ensure standardized colour measurements in this study, all samples were placed in petri dishes and pressed flat to give them a uniform shape. Colour changes were measured as the modulus of the distance vector between the initial colour values and the actual colour coordinates. This concept is named total colour difference (ΔE) (Martins and Silva 2002). Differences in perceivable colour can be analytically classified as very distinct ($\Delta E > 3$), distinct ($1.5 < \Delta E < 3$) and small difference ($1.5 < \Delta E$) (Adekunte, Tiwari, Cullen, Scannell and O'Donnell, 2010). Choi, Kim and Lee (2002) indicated that a $\Delta E > 2$ corresponds to noticeable differences in the visual perception of many products.

Total element concentrations (mass fraction) in unfortified and fortified special maize meal were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES) after microwave digestion (AOAC, 2000). A weighed amount of sample was placed in a digestion tube. Nitric acid (HNO_3) and Perchloric acid ($HClO_4$) were added and the mixture was heated for the appropriate time to achieve complete wet acid digestion of all organic matter. After digestion, the samples were dissolved in double-distilled water and then filtered using a membrane filtration. The filtrate was made up to a specific volume using the double-distilled water and used for determination of total content of zinc, iron and calcium. The sample solution was converted to an aerosol and directed into the central channel of the plasma. At its core, the inductively coupled plasma (ICP) sustains a temperature of approximately 10 000 K, so the aerosol is quickly vaporized (Hou and Jones, 2000). The minerals were quantified simultaneously using ICP-Optical Emission Spectrophotometer. The instrumentation associated with an ICP-OES system is relatively simple. Single element measurements can be performed cost-effectively with a simple monochromator/ photomultiplier tube combination, and simultaneous multi-element determinations are performed for up to seventy (70) elements with the combination of a polychromator and an array detector. The analytical performance of this system is competitive with most other inorganic analysis techniques, especially with regards to sample throughput and sensitivity (Hou and Jones, 2000).

Special maize meal oil content was determined by Soxhlet extraction method 920.39 (AOAC, 2000c). The method involves extraction of oil from the sample by use of extraction solvents such as petroleum ether. The extraction is based on the solubility of the lipids in an organic solvent (De Castro, Valcarcel and Tena, 2012). Extraction is achieved by recirculation of the solvent through the reflux system ensuring repeated contact of the solvent with the sample (De Castro and Garcia-Ayuso, 1998). The repeated contact between the sample and the fresh portions of the solvent facilitates the displacement of the transfer equilibrium (De Castro and Priego-Capote, 2010). This is done at the boiling point of the solvent for a relatively long period of time. After cooling and evaporation of the solvent, the oil is measured gravimetrically. The method is relatively simple although time consuming.

Ash content of special maize meal was determined by a muffle oven heating method 942.05 (AOAC, 2000d). Ash or more appropriately "residue on ignition" is the material remaining after oxidative combustion of water and all organic matter in food. “Ash” is therefore a measure of a food’s total mineral content. Complete ignition can be observed by the absence of black colour (due to residual carbonaceous material) in the ash residue. For this method, ignition time, ignition temperature, and type of furnace or weighing conditions are reported to influence the amount of ash content. According to this method, the muffle oven temperature and heating time for ash content determination in samples should be 600 ^{o}C and 2 hours respectively. A perfect residue after ignition of a sample would be white, with no hint of residual carbon. According to Thiex, Novotny and Crawford (2012), some official methods for ash content determination call for examination of the residue, while others do not. The current *AOAC Official Method 942.05* does not.

Moisture content of special maize meal was determined by the oven air drying method 934.01 (AOAC, 2000a). This is a standard method in moisture determination in food. It is based on drying the food sample above the boiling point of water until equilibrium moisture content is reached. The time (4 hours) and temperature (103 ^{o}C) used should ensure that all but chemically bound moisture is lost.

5.1.2 Sensory and Consumer methods

Sensory evaluation of food includes measuring, analysing, and interpreting reactions to attributes that can be directly perceived by the human senses of taste, smell, sight, touch and hearing (Meilgaard, Civille and Carr, 1999). In sensory evaluation, the key determinant of which test method should be used is the objective of the test (Lawless and Heymann, 1999). In this study, the Difference from Control (DFC) method was used for analytical sensory evaluation of stiff maize porridges prepared from iron fortified maize meal. One of the most important criterion for a satisfactory iron fortificant compound is the effect of added iron on the sensory quality of the food (Bovell-Benjamin and Guinard, 2003). The DFC method is used when the test objective is twofold; (1) to determine whether a difference exists between one or more samples and a control, and (2) to estimate the size of any such difference. Generally one sample is designated the control and all the other samples are evaluated with respect to how different each is from the control (Lawless and Heymann, 2010). The test requires 20 - 50 trained or untrained panellists, but should not consist of a mixture of the two types (Meigaard *et al.*, 2007). Training of a panel helps panellists become more familiar with the product. In this method, the control sample was presented as one of the blind coded samples and the panellists were alerted to this. The blind control helps to establish a base line for the rest of the test samples, as most blind controls will get a non-zero score due to individual variability (Lawless and Heymann, 2010). In this study, a total of 28 trained panellists evaluated the five fresh and the five stored porridges. Five stored porridge samples were evaluated first while five freshly prepared porridges were evaluated after a 15 minutes break to avoid sensory fatigue. In this study panellists identified the differences between the porridges quantitatively using a five point category scale.

Consumer sensory evaluation requires a minimum of 50 consumers (Lawless and Heymann, 1999). Consumer tests were conducted in order to determine the acceptability of the iron fortified maize porridge. Despite the importance of other factors, one of the initial hurdles to overcome in any iron fortification programme is consumer acceptance stemming from sensory effects and cost (Salgueiro, Zubillaga, Lysionek, Caro, Weill and Boccio, 2002). In this study, eighty (80) consumers (54 females and 26 males) between 18 and 74 years, who were regular consumers of stiff maize porridge (3 or more times a week) were used. To ensure that a panel consisting of only regular consumers of stiff maize porridge was recruited, a recruitment agency was hired and provided with strict guidelines on the category of consumers to recruit. By choosing consumers within these criteria it is made sure that the consumers have a frame of reference and thereby can compare the product with similar products that they have tried. It also makes sure that the consumers possess reasonable expectations on the product (Lawless and Heymann, 2010). In our evaluation form, the word “maize pap” (attached in appendix A) was used as this is how stiff maize porridge is normally referred to in households. The word “porridge” is sometimes used to refer to “thin maize porridge” hence the use of the word “maize pap” would eliminate any potential confusion during the evaluation process. A five point hedonic scale (face scale) was used to measure the overall acceptance of colour and taste of each sample. As a motivation, consumers were rewarded for their participation in the test.

5.2 Critical evaluation of colour (L* a* b*) of iron fortified maize meal and the sensory properties of stiff porridges

As expected, the proximate composition of special maize meal did agree with the South Africa Regulation for dry-milled maize products (Johnson *et al.*, 2004), however these had a lower ash content. Genetic and environmental factors have been reported to contribute to variations in maize kernel mineral content (Oikeh *et al.*, 2003). The genetic and environmental factors therefore explain the lower ash content in the maize meal.

Although whole maize kernels is a fair source of minerals, particularly iron (Bauernfeind and DeRitterk, 1991), many of these minerals, including iron are lost during milling, a process that involves the removal of the germ and outer layers of the maize kernel (Johnson *et al.*, 2004). Maize meal is a staple food in South Africa (National Food Consumption Survey, 2000). Maize meal iron fortification has been identified as potential tool to reduce the prevalence of iron deficiency among the low income earning consumers in South Africa (Department of Health, South Africa, 2003). Food fortification of staple foods with micronutrients is one of the food based strategies employed to alleviate micronutrient deficiencies in a population (WHO, 2001). In this study, the fortification process resulted in under-fortification, meaning the added iron was slightly lower than the targeted fortification level. The relatively small samples of unfortified maize meals (10 kg each) and fortificant premixes used in the study could have contributed to lower levels of iron contents than expected. The use of these relatively small quantities was in line with the number of our targeted consumers for both analytical and consumer studies as well as our limited resources. In future, fortifying larger samples could improve the distribution and hence the iron content in fortified maize meals.

Electrolytic iron significantly reduced L* and a* (more green) values in fortified maize meal when compared to maize meal fortified with multi-micronutrient mix excluding iron (control) while NaFeEDTA did not. Johnson *et al.*, (2004), reported that elemental iron powders (e.g. electrolytic iron), being black or dark grey powders may cause slight darkening of cereal flours. This explains the lower L* value in electrolytic iron fortified maize meal.

Fewer red spots were observed in unfortified maize meal as compared to the iron fortified samples. The number of red spots increased as the iron concentration was increased from 15to 35 mg iron/kg in the maize meal. However, it is important to note that the nature of the red spots is determined by the type of iron. The NaFeEDTA fortificant compound show large spots that develop quickly while elemental iron shows many small red spots that take about five minutes to appear. An increase in the number of red spots with the increase in iron concentration from 16.1 to 38.4 mg iron/kg in bread made from iron fortified flour has been reported by Nichols *et al.*, (2012). This method quantifies both added and natural iron content by reaction with the thiocyanate ion (SCN^-) from Potassium thiocyanate (KSCN) to form a blood-red coloured complex.

Fortification of maize meal with iron (electrolytic iron or NaFeEDTA) and that commercially fortified resulted in lower L* values and higher a* values (more red) compared to maize meal fortified with multi-micronutrient mix excluding iron (control sample). Commercially fortified maize meal and that fortified with electrolytic iron had higher b* values (more yellow) when compared to control. In South Africa, commercial maize fortification programmes currently involve the use of electrolytic iron (Department of Health, South Africa, 2003). Iron (Fe^{3+}) from the fortificants (NaFeEDTA or electrolytic iron) and that within the maize meal reacts with the thiocyanate iron (SCN^-) from Potassium thiocyanate (KSCN) to form a blood-red coloured complex. In order to oxidise the iron in electrolytic iron to ferric iron (Fe^{3+}), the form of iron that actively reacts with SCN^-, hydrogen peroxide (H_2O_2) and hydrogen chloride (HCl) needs to be added. Because iron in NaFeEDTA is in the ferric form, it is not necessary to add H_2O_2 and HCl in NaFeEDTA fortified maize meal. Virtually all plant food-derived iron is in the ferric (Fe^{3+}) form (Conrad and Umbreit, 2002). The addition of the iron fortificants (NaFeEDTA or electrolytic iron) or through commercial fortification resulted in more blood-red coloured complexes being formed and explains the higher a* values (more red) in iron fortified samples compared to control. The development of red colour could have led to the loss of lightness of the sample and may have affected the L* colour value. The addition of hydrogen peroxide (H_2O_2) and HCl in maize meal fortified with electrolytic iron and that commercially fortified resulted in a yellowish colour developing in the samples. This explains the higher b* values (more yellow) in these samples. The study shows a promise for the spot iron test as an inexpensive, field friendly approach for testing fortified maize meal. This approach could have a future useful role in the monitoring and evaluation process for cereal flour fortification programmes.

Electrolytic iron or NaFeEDTA 15 and 30 maize meals resulted in stiff maize porridge prepared in aluminium, cast iron and stainless steel having lower L* values when compared to porridge prepared from maize meal fortified with multi-micronutrient premix excluding iron (control sample). However for L* values, NaFeEDTA 15 porridge was closer to the control porridge. Richins *et al.*, (2008) reported that corn tortillas made from corn masa flour fortified with NaFeEDTA or electrolytic iron had lower L* values compared to corn tortillas made from corn masa flour fortified with premix excluding iron. The major polyphenols reported to be found in white maize meal are ferulic and p-coumaric acid (Pozo-Insfran *et al.*, 2006). Hurrell, (1997) suggested that polyphenols may be involved in the off-colour development of iron fortified foods. The interaction of iron with polyphenols is an oxidation/reduction reaction, where the ferric (Fe^{3+}) iron is reduced to ferrous (Fe^{2+}) and the polyphenol compounds are oxidized (Mellican *et al.*, 2003). It has been suggested that the interaction between iron and polyphenols might induce structural changes and polymerization in the polyphenols influencing their light absorption pattern and thus leading to off-colour development (Mellican *et al.,* 2003). This oxidation/reduction reaction therefore explains the lower L* values in NaFeEDTA or electrolytic iron fortified porridges when compared to porridge fortified with premix excluding iron. The b* values followed a trend that was quite similar to L* values, with NaFeEDTA 15 porridge scoring closer to the control. There did not appear to be any consistent pattern in the a* values due to iron fortification. This finding suggests that L* and b* colour values may be good predictors in perceptible sensory differences in iron fortified maize porridges.

Storage for 24 hours resulted in an increase for L* values in maize porridges prepared in aluminium, cast iron and stainless steel cookware. This finding is somehow difficult to understand considering that iron treated porridges resulted in lower L* values compared to porridges without any added iron. Storage for 24 hours should have otherwise resulted in lower L* values due to more contact time between polyphenols and iron which may have resulted in more off-colour development in porridges. Storage for 24 hours resulted in b* values that followed a trend quite similar to L*. There did not appear to be any consistent a* values due to storage. Iron treatment resulted in increase in colour difference (ΔE) for both fresh and stored maize porridges. This indicated that a possible noticeable visual change in colour due to iron treatment could be perceived by the trained panel.

Electrolytic iron or NaFeEDTA 15 and 30 maize porridges prepared in aluminium cookware were not different from control porridge (fortified with premix excluding iron) as perceived by a trained panel. NaFeEDTA 15 porridge was scored closest to the control porridge. Electrolytic iron fortification resulted in porridge that was significantly different from control porridge in terms of appearance while NaFeEDTA fortification resulted in NaFeEDTA 30 being significantly different from control in terms of flavour as perceived by a trained panel when using cast iron cookware. NaFeEDTA fortification resulted in NaFeEDTA 30 being significantly different from control porridge in terms of flavour as perceived by a trained panel when using stainless steel cookware. The differences in appearance of porridges could be due to the interactions between polyphenols (ferulic and p-coumaric acid) in maize meal and the fortificant iron ions. As mentioned earlier, Mellican *et al.*, (2003) did report that interaction between polyphenols and iron ions does lead to off-colour development in iron fortified foods. Maize also contains polyunsaturated fatty acids (PUFAs) in the form of linoleic acid (Bovell-Benjamin *et al.*, 1999). Lipid oxidation of linoleic acid in iron catalysed reactions leads to the development of hexanal, a major off-flavour compound in iron fortified maize meal porridge, which has been reported by Bovell-Benjamin *et al.*, (1999). This explains the flavour differences in iron fortified porridges when compared to control porridge.

Another possible explanation for differences of iron treated porridges when compared to control porridge in terms of appearance and/or flavour in porridges prepared in cast iron and stainless steel but not aluminium cookware could be attributed to material composition of cookware. Cast iron cookware is an alloy of mainly iron, silicon and carbon while stainless steel is an alloy of iron, chromium and carbon (Tilley, 2004). Cast iron and stainless steel cookware have been shown to leach iron ions (Fe^{3+}/Fe^{2+}) during the cooking process and the leaching of these iron ions is said to be pH dependent (Bi, 1995; Kuligowski and Halperin, 1992; Verissimo *et al.*, 2006). Kuligowski and Halperin (1992) reported that when 5 % acetic acid was boiled for 5 minutes in cast iron and stainless steel cookware, the corrosion of cast iron and stainless steel cookware measured by the quantity of iron in water was 3.5 mg iron/kg and 2.9 mg iron/kg respectively. The leached iron ions (Fe^{3+}/Fe^{2+}) could have contributed additional iron ions leading to more appearance and flavour changes in the maize porridges. Further studies on the actual amount of iron ions (mg iron/kg porridge) that leaches into the maize porridge during cooking should be carried out in the future. Bioavailability of iron in the fortified maize porridges should also be carried out in the future.

Storage for 24 hours was significant for appearance of porridge prepared in aluminium cookware. This is somehow difficult to understand as one would have expected this significance to appear in either cast iron or stainless steel pot where evidence of leaching iron ions has been demonstrated. The leached iron ions have the potential to contribute to reactions that lead to additional colour and flavour changes in the porridge. Another point to note here is that the stored porridges samples were portioned into glass ramekins covered with plastic lids and put on coded trays in a refrigerator prior to sensory evaluation. However, refrigerating the maize porridges in their respective cookware could have a given a better understanding of effect of pot type on the porridges.

Poor consumer acceptance, unacceptable taste and discolouration of iron fortified foods have frequently been listed as causes of unsuccessful iron fortification programmes (Bovell-Benjamin *et al.*, 2003). Maize porridge fortified with electrolytic iron was liked slightly more than NaFeEDTA fortified porridge in terms of taste when cast iron cookware was used. Possible lipid oxidation of linoleic acid by NaFeEDTA leading to the development of hexanal, a major off-flavour compound indicator could explain why porridge fortified with electrolytic iron was liked slightly more. NaFeEDTA, a slightly water soluble compound could have contributed to greater flavour changes in maize porridge than the water insoluble and very stable electrolytic iron (Hurrell, 1997).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Electrolytic iron had a significant effect on the colour of fortified maize meal but NaFeEDTA did not. Electrolytic iron (35 mg iron/kg) forms part of the currently legislated multi-micronutrient fortificant premix while NaFeEDTA (15 mg iron/kg) is the proposed replacement.

The spot iron test performed acceptably as a general indicator of the presence or absence and distribution of added iron in maize meal. However, it is important to note that the nature of the red spots is determined by the type of iron fortificant used. This suggests that this type of a field friendly and inexpensive method for testing maize meal could have a useful role in monitoring and evaluation process for fortification programmes.

All iron fortificants significantly affected the L* a* and b* colour values of fortified porridges when compared to control porridge, using all three types of cookware (aluminium, cast iron and stainless steel). Storing the porridges for 24 hours in a refrigerator also had a significant effect on the colour of porridges. These differences in colour of maize porridges suggested that a possible noticeable visual colour difference due to iron treatment could be perceived through sensory evaluation.

However, there were no significant differences in the sensory properties (appearance and flavour) of porridges fortified with multi-micronutrient premix containing NaFeEDTA at 15 mg iron/kg and electrolytic iron at 35 mg/kg maize meal in all three types of cookware. Even an excess amount of NaFeEDTA at 30 mg iron/kg was not detected sensorially as a change. This finding suggests that a change from using electrolytic iron to using NaFeEDTA will not lead to changes in the appearance and flavour of maize porridges. However, when considering acceptability studies, excess amounts of NaFeEDTA (beyond what is proposed) might lead to acceptability (taste) problems if porridge is prepared in cast iron cookware.

NaFeEDTA is more expensive than electrolytic iron. However, NaFeEDTA can be applied at a significantly lower dosage level than that of electrolytic iron in high extraction maize meal with an equivalent bioavailability effect. The more bioavailable form of iron (NaFeEDTA) could be a viable option to reduce prevalence of iron deficiency in South Africa.

Further studies on the bioavailability of iron specifically in maize porridges are required in future to evaluate the two different iron fortificant compounds in application.

CHAPTER 7: REFERENCES

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8. APPENDICES

8.1 Appendix A

Interview sheet for consumer sensory evaluation of stiff maize porridge

SET NUMBER……………….

Interview schedule: Maize pap Consumer Sensory Evaluation

1. Good day, my name is [...] and I am glad that you are here.
2. Before we start I would like to explain to you the reason why you are here today.

3.	**I assume that you eat maize pap very often?**	**Yes** **1**	**No** **2**		**3.**
4.	**On average, on how many days of the week (7 days) do you eat maize pap?** Listen to answer , prompt if no response: Record the number of days		**Days**		**4.**

5. That is very good, because today we need the opinion of persons that know maize pap very well.

6.	**Did you know that iron (Fe), a micronutrient, has to be added to maize meal in South Africa**	**Yes** **1**	**No** **2**		**6.**

6.1 The reason why iron **(a micronutrient)** is added to maize meal is to ensure that consumers that eat maize meal get enough iron which is needed by the human body. If you do not eat enough food with iron you can become very ill.

7. Iron can be added to maize meal in different ways. What we want to determine with your help today is to find out if the way the iron is added has an effect on the way you like maize pap to look and taste.

8. You will be asked to look at and taste four small portions of maize pap and tell me how much you like or dislike them. At the end I will ask you some questions about the maize meal you buy and your eating habits.

9. If you do not feel comfortable to answer any of the questions, that is ok. You do not have to answer if you do not want to.

10. **THERE IS NO RIGHT OR WRONG ANSWERS, ONLY YOUR PERSONAL, HONEST OPINION MATTERS.**

11. At the end of the session I will ask your permission to include the answers that you gave in our data set.

12.	Do you have any questions to ask before we start?	**Yes** **1**	**No** **2**		**12.**
12.1 Record any questions from consumers that may be of interest to the researchers.					**12.1**

13. I will now serve you the maize porridges (pap), one at a time for you to look at and taste. **Please drink water before you start tasting and also in between each sample.**

14. Each time I will ask you how much you like or dislike the product, you will have to show me which face (SHOW CARD WITH SCALE) best describes HOW MUCH YOU LIKE OR DISLIKE THE PRODUCT.**NB: <u>Interviewer must then explain the meaning of the faces on the scale.</u>**

TRAY NUMBER.......................

15. Please open the lid. Before you taste, LOOK at the product,
HOW MUCH DO YOU LIKE OR DISLIKE THE COLOUR OF THIS PRODUCT?
Do you have any comments on the COLOUR of the product?

16. Please EAT some of the product using your hands.
HOW MUCH DO YOU LIKE OR DISLIKE THE TASTE OF THE PRODUCT?
Do you have any comments on the TASTE of the product?

Sample code....................									
Colour					**Taste**				
1	***2***	***3***	***4***	***5***	***1***	***2***	***3***	***4***	***5***
Comments					**Comments**				

Sample code....................									
Colour					**Taste**				
1	***2***	***3***	***4***	***5***	***1***	***2***	***3***	***4***	***5***
Comments					**Comments**				

Sample code....................									
Colour					**Taste**				
1	***2***	***3***	***4***	***5***	***1***	***2***	***3***	***4***	***5***
Comments					**Comments**				

Sample code....................									
Colour					**Taste**				
1	***2***	***3***	***4***	***5***	***1***	***2***	***3***	***4***	***5***
Comments					**Comments**				

Questions to be asked at the end of the evaluation

17.	**Do you think it is a GOOD or BAD idea to add iron (Fe) to maize meal?**		**17**
	Good idea	**1**	
	Bad idea:	**2**	
	I do not know	**3**	
17.1	**Record any comments that may be important for the researcher**		**17.1**

18.	**Who normally buys the maize meal in your house?**		**18**
	The participant	**1**	
	Someone else, specify:	**2**	
18.1			**18.1**

19.	**If you can remember, tell me which brand or brands of maize meal do you (or someone else) PREFER to buy or to use in your house?** ***Listen to answer, do not read the names***		**19.**
	No brand mentioned spontaneously	**1**	
	White star	**2**	
	Ace	**3**	
	Iwisa	**4**	
	Impala	**5**	
	Nyala	**6**	
	If any other, specify	**7**	
19.1			

20.	**Who MOSTLY prepares the maize pap at home?**		**20.**
	The participant	**1**	
	Someone else, specify:	**2**	
20.1			

21.	**Do you PREFER to eat maize pap COLD or HOT/WARM?**		**21.**
	Cold	**1**	
	Hot/Warm	**2**	
	I do not know	**3**	
	I eat it both ways	**4**	

22.	**Record gender of consumer**	**Male** **1**	**Female** **2**		**22.**

23.	**In what year were you born?**					**23.**

24. **Thank you for your time to help with the research.** All the answers that you gave will be treated in a confidential manner. Your answers will not be linked to your name in any way.

25.	**Do you give permission for us to useyour answers for our research**	**Yes** **1**	**No** **2**		**25.**

26. Respondent Consent

I, ____ (name and surname)

<u>consent</u> that my answers may be used for the research project as explained.

____
Signature

27. Interviewer Section

I, ____ (interviewer full name and surname) hereby declare that I have interviewed the respondent and recorded the responses as accurately as possible.

____
Signature

Date (Year/Month/Day)........................../....................../.........................

8.2 Appendix B (Comments are presented verbatim)

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron cookware

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
1	NaFeEDTA		Tastes like soft porridge, under-cooked as well
1	Electrolytic iron		It is very tasty and filling
2	NaFeEDTA	It looks more darker	It taste ok
2	Electrolytic iron	It looks dark	It just fine
3	NaFeEDTA	Grey to brown colour	Uncooked flavour
3	Electrolytic iron	It looks proper	Nice flavour, good level of salt
4	NaFeEDTA		Tastes powdery
4	Electrolytic iron	Looks darker	Tastes like milk sugar and salt was added
5	NaFeEDTA	Colour not apetising	Tastes raw
5	Electrolytic iron	Looks grey	Would not mind eating it
6	NaFeEDTA	Small round things. Normally uniform white, with no bits in it	It's better than the previous, nice and soft
6	Electrolytic iron	No No	Tastes uncooked. Can still taste the maize
7	NaFeEDTA	Dull white. Not sure what colour it is	Likes taste but more salty
7	Electrolytic iron	Dark colour	Nice, but a little soft
8	NaFeEDTA	Because I prefer white colour pap	Taste solid in the mouth
8	Electrolytic iron	She compare the colour to the first one	It taste good, I like it as the first one
9	NaFeEDTA	Not as white as he would like	Too soft though the taste is fine
9	Electrolytic iron	More whitish than all the others	It is a bit firmer in texture, and the taste is fine
10	NaFeEDTA		Little bit salty. Taste slightly different
10	Electrolytic iron		Not the usual pap I would eat
11	NaFeEDTA		Tastes salty
11	Electrolytic iron		Tastes salty, the saltiness fades away as you swallow
12	NaFeEDTA	Its too dark	It is not nice
12	Electrolytic iron	It looks dark. I am used to white pap	it tastes more like mielie bread
13	NaFeEDTA	it looks dull	has after taste, too salty
13	Electrolytic iron		very nice, tastes like normal pap
14	NaFeEDTA		very salty
14	Electrolytic iron		
15	NaFeEDTA		It's nice
15	Electrolytic iron	Has spots	Sour
16	NaFeEDTA	Looks like braai pap; it has a rough look	It does not feel as rough as braai pap; it has a smooth mouthfeel; it has no aftertaste
16	Electrolytic iron	It looks like it was mixed with brown maize meal	It is nice; can eat it a lot

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
17	NaFeEDTA	Usually the papa I make is more white	Taste like baby porridge and salty
17	Electrolytic iron	Darker	No reason but I think they took more time to cook or they added something that we don't usually use
18	NaFeEDTA	Looks dark, like they are lumps. Colour looks too grey	Tastes good initially, but has an unpleasant metallic aftertaste
18	Electrolytic iron	Looks hard, old, colour is a bit grey	Tastes nice like there are spices in it. Has a nice level of saltiness
19	NaFeEDTA	Looks dark, clay	Proper pap texture (braai pap)
19	Electrolytic iron	Looks grainy	Too soft
20	NaFeEDTA	Brownish -yellow	It has after taste
20	Electrolytic iron	It looks yellow	Not fine (mouth feel) but tastes really good
21	NaFeEDTA		
21	Electrolytic iron		Tastes salty
22	NaFeEDTA	Darker colour	Edible, not bad at all
22	Electrolytic iron	Looks like the pap I am used to	
23	NaFeEDTA	It looks dark	I can eat this
23	Electrolytic iron	It does not look bright	It tastes floury
24	NaFeEDTA		salty
24	Electrolytic iron		salty
25	NaFeEDTA		
25	Electrolytic iron		
26	NaFeEDTA	looks delicious	
26	Electrolytic iron	Would not mind finishing it off	
27	NaFeEDTA	It's got the worst taste, colour is not attractive	It's not nice, tastes very salty, pap tastes uncooked
27	Electrolytic iron	Yellow, dark , do not like because it's not white	It doesn't like taste , it's a bit salty, doesn't taste like the one we eat at home
28	NaFeEDTA	Has little brown colour	Can be eaten without condiments
28	Electrolytic iron	It looks fawn	Tastes like pap we cook at home
29	NaFeEDTA	Its not as white as I want, looks greyish	Does not taste good, tastes very uncooked, and is too soft
29	Electrolytic iron	Looks like pap does when it is mixed with butter	It is fine, the salt level is ok but it is soft and tastes better than all the other samples
30	NaFeEDTA	Grey colour	Is taste a bit nice, compare to my own pap
30	Electrolytic iron	No reason	No reason
31	NaFeEDTA	more darker	bit dry but good, almost like porridge
31	Electrolytic iron	similar to normal pap	tastes like pap he always eats

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
32	NaFeEDTA		
32	Electrolytic iron		
33	NaFeEDTA		It tastes like porridge but do not like the way it was prepared
33	Electrolytic iron		
34	NaFeEDTA		
34	Electrolytic iron		
35	NaFeEDTA		salty
35	Electrolytic iron	Its brown	
36	NaFeEDTA	Cream white colour - looks delicious	Tastes like pap he always eat at home
36	Electrolytic iron	Cream white colour - looks delicious	It is delicious
37	NaFeEDTA	It little bit darker	
37	Electrolytic iron		Too salty
38	NaFeEDTA	She asked if it the same maize meal powder used as the first sample	Does not understand the taste
38	Electrolytic iron	Does not like colour	It's nice and not salty
39	NaFeEDTA		Tastes very nice, just don't like the colour as well as I like the taste
39	Electrolytic iron	Not as white as wanted	Tastes very good
40	NaFeEDTA	Like the one she cook	Nice taste
40	Electrolytic iron	Brighter	Taste like my pap
41	NaFeEDTA	Black spots, I don’t know whether they should be there	Don’t feel it
41	Electrolytic iron	White	Normal pap
42	NaFeEDTA		
42	Electrolytic iron		It is not salty, similar to previous sample
43	NaFeEDTA	It is bright	
43	Electrolytic iron	It looks brown	It is nice (smooth)
44	NaFeEDTA	looks un cooked brown grayish colour	Tastes uncooked
44	Electrolytic iron	Dark grey colour and does not look appetising	Too salty
45	NaFeEDTA	Looks burnt	Floury
45	Electrolytic iron		
46	NaFeEDTA	It looks lighter/brighter than the first sample	It is bland
46	Electrolytic iron		It tastes like normal pap

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
47	NaFeEDTA		Tastes fine
47	Electrolytic iron	Bit dull	Not good
48	NaFeEDTA	Yellow, its fine	Tastes good
48	Electrolytic iron	Dark-grey, it's not white	Not sure of the taste, not so nice
49	NaFeEDTA	Looks a bit yellow	Not well cooked
49	Electrolytic iron		Tastes very good
50	NaFeEDTA	Grey colour	It's not good
50	Electrolytic iron	Look grey	Tasteless
51	NaFeEDTA	Dark in colour, like white pap	Salty
51	Electrolytic iron		Tastes right
52	NaFeEDTA		A bit salty
52	Electrolytic iron	It looks the same as previous sample	It is soft
53	NaFeEDTA	Looks like it has been cooked for a long time	
53	Electrolytic iron		
54	NaFeEDTA	Not normal	
54	Electrolytic iron	Not normal	
55	NaFeEDTA	It looks familiar	Good texture, taste familiar, similar to pap we buy at home
55	Electrolytic iron		Grainy texture
56	NaFeEDTA		
56	Electrolytic iron		Tastes like super sun maize meal
57	NaFeEDTA	Has normal colour but no good vision	Good hard texture and slightly sour
57	Electrolytic iron	Has normal colour but no good vision	Too soft and smooth
58	NaFeEDTA	Colour does not look like pap, does not like it	Soft an nice
58	Electrolytic iron	Bluish colour	Does not taste like cooked
59	NaFeEDTA	Its dark coloured	Does not taste well cooked
59	Electrolytic iron	It looks old, it is dark in colour, rather than white as desired	It is salty in taste
60	NaFeEDTA	Brown colour	Taste like sour pap
60	Electrolytic iron	darker	A bit bitter
61	NaFeEDTA	Looks as if cooked in poijie pot	Salty-similar to samp
61	Electrolytic iron	Looks as if cooked in poijie pot	Iwisa morning pap - tastes similar

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
62	NaFeEDTA	The colour is different from the usual pap	
62	Electrolytic iron		
63	NaFeEDTA		Not properly cooked
63	Electrolytic iron		Softer than the pap I am used too, not overcooked
64	NaFeEDTA	It looks raw	Tastes like pap I eat at home
64	Electrolytic iron		Tastes sour
65	NaFeEDTA		
65	Electrolytic iron		
66	NaFeEDTA		
66	Electrolytic iron		
67	NaFeEDTA	Not grey/dark, though a bit creamier than desired. It is fine	Liked most than all others. Not too salty but is actually the right taste balance. Not too bad
67	Electrolytic iron	Colour is basically the same as he is used to	It is salty and not too sour
68	NaFeEDTA	Grey colour	Tasteless
68	Electrolytic iron	Look smooth	Very smooth
69	NaFeEDTA	In between white and dark	It's not bad
69	Electrolytic iron	It's almost white	It's ok
70	NaFeEDTA	Creamy look, almost grey	Soft texture, bitter/sweeter taste
70	Electrolytic iron	Inviting colour-white grey	Good taste, not too salty
71	NaFeEDTA	Cream white	Too salty
71	Electrolytic iron		A little bit sweet
72	NaFeEDTA	It looks grey	
72	Electrolytic iron	It looks like it has over stayed	
73	NaFeEDTA	Looks normal	
73	Electrolytic iron	It does not look appetising	
74	NaFeEDTA		
74	Electrolytic iron		
75	NaFeEDTA	Looks like pap, very white and fluffy soft	Taste is fine, needs gravy, similar to pap I consume at home
75	Electrolytic iron	Looks very dark and damp	Smooth, not very tasty, needs some flavour, does not stick to teeth
76	NaFeEDTA		Texture similar to pap I eat at home
76	Electrolytic iron		Soft texture

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in cast iron cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
77	NaFeEDTA	It looked different but ok	Almost taste like phuthu
77	Electrolytic iron		Tastes like the second sample, A bit too bland
78	NaFeEDTA	Looks fine, but looks grainy, and does not the graininess	It's nice, it's like the one I ate at home
78	Electrolytic iron	Right colour, normal pap colour	Mouth feel - soft like
79	NaFeEDTA	Darker colour	Not well cooked
79	Electrolytic iron	Good white colour	slightly too salty but edible
80	NaFeEDTA		
80	Electrolytic iron	Worse really dark	Tasteless

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in stainless steel cookware

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
1	NaFeEDTA		It is not filling
1	Electrolytic iron		Tastes salty
2	NaFeEDTA	It is not white as I am used to, it does not look appealing	It tastes salty. At home we do not eat pap with salt
2	Electrolytic iron	It looks alright and the lightest among the four samples	It tastes good. It's similar to the pap I am used to
3	NaFeEDTA	Almost yellow	Nice taste
3	Electrolytic iron	Grey colour	Salty flavour
4	NaFeEDTA		Tastes normal
4	Electrolytic iron	Looks normal	Salty. Tastes like milk was added
5	NaFeEDTA	Just ok	It's really good
5	Electrolytic iron	Colour not appetising	Its eatable
6	NaFeEDTA	Not cooked	Not so good as normal pap, not nice at all
6	Electrolytic iron	Much better compared to the others	Better taste. If had butter, taste would be better
7	NaFeEDTA	Dark colour	Hard texture
7	Electrolytic iron	Darker colour, but generally the same	Burnt taste
8	NaFeEDTA	The colour is too white	Is taste more salty
8	Electrolytic iron	Is look like the normal pap	I am used to this kind of pap
9	NaFeEDTA	Not as white as he would like	Taste is not as good as the sample before
9	Electrolytic iron	Not as white as he would like	Taste is very fine
10	NaFeEDTA		Tastes like unusual powder
10	Electrolytic iron	Look softer	More like pap I am used to
11	NaFeEDTA		Tastes under cooked
11	Electrolytic iron		Tastes under cooked
12	NaFeEDTA	It is not a bad colour	It is weird
12	Electrolytic iron	It looks a bit greyish	It looks weird
13	NaFeEDTA		No aftertaste or graininess
13	Electrolytic iron		Feels grainy and has after taste
14	NaFeEDTA		
14	Electrolytic iron		A bit sweet
15	NaFeEDTA		Aftertaste
15	Electrolytic iron		Sticky
16	NaFeEDTA	It looks like mashed potato; it is white	Not salty; it has a smooth mouthfeel; it will go well with other food
16	Electrolytic iron	It looks different from the normal pap	Great aftertaste; a little salty

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in stainless steel cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
17	NaFeEDTA	White colour	Seem like there is something they didn't add to the pap like salt
17	Electrolytic iron	Little whiter, as I like	Is like the pap I made
18	NaFeEDTA	Looks properly made, not dry, does not look burnt, not as white as she would have liked	Not too hard, no lumps, taste good, like there are some spices in it
18	Electrolytic iron	Looks white, not grey, and looks soft	Tastes plain, with slightly uncooked tasting pieces
19	NaFeEDTA	Looks softer	Kind bitter
19	Electrolytic iron	Looks good, appetising like pap I am used to	Porridge taste not like pap
20	NaFeEDTA	The colour is ok, not too yellow or too white	Tastes ok, not too soft not too hard
20	Electrolytic iron	It looks a bit yellow and not white	Feels really soft, tastes really good
21	NaFeEDTA		
21	Electrolytic iron		It is filling
22	NaFeEDTA		After taste. Not used to
22	Electrolytic iron	Perfect colour	Nice, just like the pap I prepare at home
23	NaFeEDTA	It does not look alright	It tastes the same as the previous pap
23	Electrolytic iron	It is bright	It is eatable
24	NaFeEDTA		Salty. Not properly cooked
24	Electrolytic iron		Tastes floury and salty
25	NaFeEDTA		Tastes similar to the first sample
25	Electrolytic iron		
26	NaFeEDTA	Looks unappetizing	a bit salty
26	Electrolytic iron	very nice, looks well cooked	very nice, though salty
27	NaFeEDTA	Likes it because its whiter	Tastes nicer than the first sample
27	Electrolytic iron	The white is not very white	Tastes nicer than the first sample
28	NaFeEDTA	Has cream white colour	Nice flavour, good level of salt
28	Electrolytic iron	White colour	Nice flavour
29	NaFeEDTA	Looks like the familiar colour of pap	Tastes almost the same as the previous sample. It is thicker, not as salty as the last sample
29	Electrolytic iron	It’s not as white as I want. It’s a colour I'm not used to	Tastes familiar and is good. Really soft
30	NaFeEDTA	Don’t look proper cook and has grey colour	Is like porridge
30	Electrolytic iron	Look like my own pap	Taste nice
31	NaFeEDTA		Tastes like pap he always eats
31	Electrolytic iron	Bit darker	Better taste
32	NaFeEDTA		
32	Electrolytic iron		

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in stainless steel cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
33	NaFeEDTA		
33	Electrolytic iron	It looks similar to the pap we eat at home	
34	NaFeEDTA		It is not cooked
34	Electrolytic iron		It is cooked
35	NaFeEDTA		
35	Electrolytic iron		
36	NaFeEDTA	White colour	Good taste
36	Electrolytic iron	Cream white colour - looks good	It has after taste
37	NaFeEDTA	It looks lighter than the first two	Not too different
37	Electrolytic iron	It little bit darker	It is the same as the first sample
38	NaFeEDTA	It's not white that’s why I don’t like it	Its salty a bit, does not like salt in pap
38	Electrolytic iron	Its white	Likes a lot
39	NaFeEDTA	Not as white as desired	Taste is just alright
39	Electrolytic iron		Taste is great
40	NaFeEDTA	No reason	Taste a bit like my pap
40	Electrolytic iron	Too dark	Smooth like flour
41	NaFeEDTA		Just as nice as the last one
41	Electrolytic iron	Looks better that first one	Nice, but not to my satisfaction
42	NaFeEDTA	It looks like dumpling	Tastes salty
42	Electrolytic iron	Similar to the one I eat at home	
43	NaFeEDTA		It is nicer than the first one
43	Electrolytic iron	It looks white	It is nice
44	NaFeEDTA	Nice white colour for pap	Just enough salt, soft and cooked
44	Electrolytic iron	Cream white colour	Very salty
45	NaFeEDTA		
45	Electrolytic iron		
46	NaFeEDTA	It has a dull colour and I am not used to it	It tastes different but good
46	Electrolytic iron	It looks white, like the usual colour of maize pap	
47	NaFeEDTA	Bit dull	
47	Electrolytic iron	Colour is fine	
48	NaFeEDTA	Dark -cream/white, greyish	It's alright
48	Electrolytic iron	White-grey	Fine, tastes nice

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in stainless steel cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
49	NaFeEDTA		Texture is firmer, and taste is great
49	Electrolytic iron		Too soft, but tastes good
50	NaFeEDTA		
50	Electrolytic iron	Nice colour similar to what I normally cook	Like my own pap
51	NaFeEDTA		Bitter , don't like it
51	Electrolytic iron	Right colour	Right taste
52	NaFeEDTA	It looks like the two previous samples	Feels soft in the mouth
52	Electrolytic iron	It looks burnt	It has after taste
53	NaFeEDTA		Less salty and good
53	Electrolytic iron	Not sure	
54	NaFeEDTA	Darker than normal	
54	Electrolytic iron	n/a	
55	NaFeEDTA	It looks familiar	Tastes under-cooked
55	Electrolytic iron		Has a bit of after taste
56	NaFeEDTA		
56	Electrolytic iron		Tastes like super sun maize meal pap
57	NaFeEDTA	Has normal colour but no good vision	Slightly sour taste
57	Electrolytic iron	Has normal colour but no good vision	Too soft, good to eat with milk
58	NaFeEDTA	Grey, not that white	Soft, porridge like, delicious, does taste like pap
58	Electrolytic iron	Bluish colour	Not soft, but tastes like normal pap we eat at home
59	NaFeEDTA	Its white enough	It's the taste of pap that is very familiar and pleasant
59	Electrolytic iron	Looks like the first sample	Its salty like the first sample
60	NaFeEDTA	Like the colour	Neutral taste
60	Electrolytic iron	Nice creamy	Nice in taste
61	NaFeEDTA	Looks as if cooked in poijie pot	Tastes like samp
61	Electrolytic iron	Looks as if cooked in poijie pot	Salty- sticks t mouth
62	NaFeEDTA		
62	Electrolytic iron	It looks better than the rest	
63	NaFeEDTA		Bit soft and a sour taste
63	Electrolytic iron		Normal pap for me
64	NaFeEDTA		Sour but nice
64	Electrolytic iron	It looks raw	Soft

Consumer comments on acceptability (colour and taste) of stiff maize meal porridge prepared from maize meal fortified with NaFeEDTA at 30 mg iron/kg maize meal or electrolytic iron at 35 mg iron/kg maize meal and prepared in stainless steel cookware (continued)

Consumers	Iron fortificant compound	Comment on colour of maize porridge	Comments on taste of maize porridge
65	NaFeEDTA		
65	Electrolytic iron		Salty
66	NaFeEDTA		It tastes like maltabela
66	Electrolytic iron		Almost similar to super sun and Cup final
67	NaFeEDTA	Looks creamier in colour than previous sample	Less salty than previous sample
67	Electrolytic iron	Looks exactly the same as last sample	Tastes the same as the first sample
68	NaFeEDTA	No reason	No reason
68	Electrolytic iron	similar to the pap I cook at home	salty
69	NaFeEDTA	It’s almost white	It’s a bit sweet
69	Electrolytic iron	It's not very white	Its tasty
70	NaFeEDTA	grey- white colour, looks good	soft texture, tasteless
70	Electrolytic iron	It has brownish colour	Good texture, nice flavour
71	NaFeEDTA	White	
71	Electrolytic iron		Metallic taste, rust
72	NaFeEDTA	It looks like pap I normally eat at home	
72	Electrolytic iron		
73	NaFeEDTA		Not what I am used to
73	Electrolytic iron	Looks interesting	Cream taste
74	NaFeEDTA		
74	Electrolytic iron		
75	NaFeEDTA	Smells like porridge, looks hard, looks greyish	Sticky, it ha after taste
75	Electrolytic iron	Looks darkish, not completely white	Not toobad, needs condiment, looks like normal pap
76	NaFeEDTA		Has more taste compared to others
76	Electrolytic iron		Soft texture
77	NaFeEDTA	Looks like the previous samples	Taste is good, better than the last sample
77	Electrolytic iron	It looks just like the first sample	Does not taste a nice, a bit too bland
78	NaFeEDTA	Does not the yellow cream colour of the porridge	Taste like dough, like it very much
78	Electrolytic iron		It's just bland
79	NaFeEDTA	good white colour	tastes like pap we cook at home
79	Electrolytic iron	Light grey colour	Nice taste
80	NaFeEDTA	Look like the normal	Taste like my mother pap
80	Electrolytic iron	Darker	not really terrible