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# Bioavailability of iron from fortified maize using stable isotope techniques

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## AFRIKAANSE TITEL

Die biobeskikbaarheid van yster vanaf gefortifiseerde mieliemeel deur die gebruik van stabiele isotoop tegnieke.

## OPSOMMING


### Agtergrond

Die hoë voorkoms van ystertekort en anemie onder Suid-Afrikaanse kinders beklemtoon die noodsaaklikheid van ysterfortifisering, veral deur middel van 'n hoogs biobeskikbare ysterverbinding. Fortifisering van stapelvoedsels is 'n geskikte strategie vir die voorsiening van bykomstige yster aan bevolkingsgroepe wat geneig is om tekorte te toon. In Suid-Afrika is dit verpligtend om yster, sowel as ander mikronutriënte by mieliemeel en meelblom te voeg. Elementele yster, veral elektrolitiese yster, is tans die keuse wat voorkeur geniet, maar ander verbindings wat moontlik meer effektief is om ystertekort die hoof te bied, word oorweeg.

### Doelstellings

Die doelstelling van hierdie studie was om inligting oor die biobeskikbaarheid van ysterfumaraat en NaFeEDTA vanaf mieliemeel in jong kinders te voorsien, wat terselfdertyd sou kon help om 'n biobeskikbare alternatief te kies vir elektrolitiese yster in die Suid-Afrikaanse Nasionale Voedselfortifiseringsprogram.

### Metode

'n Ewekansig parallelle studie-ontwerp is gebruik waar elk van die 2 groepe verder ewekansig verdeel is om een van twee dieetvoorskrifte in 'n oorkruis ontwerp te ontvang waarin elke kind as sy/haar eie kontrole dien. Yster se biobeskikbaarheid is 15 dae ná inname met 'n stabiele-isotoop tegniek, wat op rooibloedsel inkorporasie gebaseer is, gemeet.

### Resultate

Die gemiddelde absorpsie van yster afkomstig van NaFeEDTA en ysterfumaraat vanuit die mieliemeelpap was 11.5% en 9.29% onderskeidelik. NaFeEDTA en ysterfumaraat is ewe voldoende biobeskikbaar vanuit 'n mieliegebaseerde maaltyd ryk aan fitaat.

**Gevolgtrekking**

Beide NaFeEDTA en ysterfumaraat sal 'n fisiologies belangrike hoeveelheid yster kan lewer sou hulle elektrolitiese yster vervang as ysterfortifikant in mieliemeelfortifisering. Die finale keuse tussen ysterfumaraat en NaFeEDTA as alternatiewe ysterfortifikant sal op faktore soos tegniese verenigbaarheid, biobeskikbaarheid, relatiewe koste en organoleptiese eienskappe berus.

**Sleutelwoorde:** ysterbiobeskikbaarheid, stabiele isotope, ysterfumaraat, NaFeEDTA, sodium yster etileen diamien tetra asetaat, mieliemeel, voedselfortifisering

## ABSTRACT

### Background

The high prevalence of iron deficiency and anaemia among South African children highlights the need for iron fortification, especially with a highly bioavailable iron compound. Fortification of staple foods is an adequate strategy to provide additional iron to populations at risk. In South Africa it is mandatory to fortify maize meal and wheat flour with iron, as well as other micronutrients. Elemental iron, specifically electrolytic iron, is currently the preferred choice but other compounds that might be more effective in alleviating iron deficiency are under consideration.

### Objectives

The objective of this study was to provide information about the bioavailability of ferrous fumarate and NaFeEDTA from maize meal porridge in young children, which would assist in selecting a bioavailable alternative to electrolytic iron in the South African National Food Fortification Programme.

### Methods

A randomized parallel study design was used, with each of the 2 groups further randomised to receive either one of two test regimens in a crossover design in which each child acted as his/her own control. Iron bioavailability was measured with a stable-isotope technique based on erythrocyte incorporation 15 days after intake.

### Results

The mean absorption of iron from NaFeEDTA and ferrous fumarate from the maize porridge meal was 11.5% and 9.29% respectively. NaFeEDTA and ferrous fumarate are both sufficiently bioavailable from a maize based meal rich in phytates.

### Conclusion

Both NaFeEDTA and ferrous fumarate would provide a physiologically important amount of iron should they replace electrolytic iron as fortificant in maize flour fortification. The final choice between ferrous fumarate and NaFeEDTA as when it comes to finding the alternative iron fortificant will depend on factors such as technical compatibility, bioavailability, relative cost and organoleptic characteristics.

**Keywords**: Iron bioavailability, stable isotopes, ferrous fumarate, NaFeEDTA, sodium iron ethylenediaminetetraacetec acid, maize meal, food fortification

## ABBREVIATIONS

| | |
|---|---|
| AI | Adequate intake |
| DRIs | Dietary reference intakes |
| EAR | Estimated average requirement |
| EDTA | Ethylenediaminetetraacetic acid |
| FAO/WHO | Food and Agriculture Organisation / World Health Organisation |
| FeFum | Ferrous fumarate |
| $FeSO_4$ | Ferrous sulfate |
| IAEA | International Atomic Energy Agency |
| ID | Iron deficiency |
| IDA | Iron deficiency anaemia |
| NaFeEDTA | Sodium iron ethylenediaminetetraacetic acid |
| NFCS | National Food Consumption Survey |
| NFFP | National Food Fortification Programme |
| RBV | Relative bioavailability |
| RDA | Recommended dietary allowances |
| SAVACG | South African Vitamin A Consultive Group |
| SD | Standard deviation |
| UL | Upper intake level |
| UNICEF | United Nations Children's Fund |

# CONTENTS

## CHAPTER 4: GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

# PREFACE

## INTRODUCTION

During the past two decades, substantial progress has been made in developing and improving stable isotope techniques to study mineral and trace element metabolism in humans, establishing them as powerful tools in nutrition research. Stable isotope techniques are preferable to radio isotopic techniques, and their use in humans is absolutely safe (IAEA, 2001).

The International Atomic Energy Agency (IAEA) has been supporting activities in the field of nutrition since the 1970s. In 1987, it established a sub-programme on nutrition related research, with a focus on applications of isotopic techniques for measuring nutrients in foods and in the human body. In 1992, the IAEA increased its efforts to bridge the gap between industrialised and developing countries' access to isotopic techniques. The IAEA is contributing to efforts to prevent malnutrition, infectious disease and environmental pollutants and to identify effective strategies in nutrition intervention schemes, particularly among vulnerable groups in developing regions around the world (IAEA, 2001).

A Technical Cooperation (TC) project was approved in 2002 to use stable isotopes to evaluate the proposed food fortification project in South Africa. The project is a four way partnership between the Nutrition Department of the North-West University (Potchefstroom Campus), the South African Government (Lynn Moeng – Directorate Nutrition), Baylor College of Medicine (Prof. Steve Abrams & Dr. Dave Hilmers, Houston, USA) and the IAEA in co-operation with the Nuclear Energy Corporation of South Africa.

The original TC project as proposed and approved included a component of directly evaluating the bioavailability of the iron in the fortified maize meal. The South African National Food Fortification programme has chosen elemental electrolytic iron as the iron fortificant. However, it is not technically feasible to evaluate the bioavailability of electrolytic iron by means of stable isotope techniques. This is because the stable isotope of iron, as well as radioisotopes of iron, cannot be made in a form identical to the iron that is used in the fortification process. The isotopic iron will always be purer and of smaller particle size than that used commercially.

Since the TC project was already established and the direct evaluation of the bioavailability of electrolytic iron not possible, other important questions regarding the iron fortificant needed to be answered: Is the electrolytic iron being used in the National Food Fortification Programme efficient to manage the risk of iron deficiency in the population? Should an alternative iron compound be considered to replace electrolytic iron as the iron fortificant?

The elemental iron powders currently available for commercial use are significantly less well absorbed compared to ferrous sulfate. Hoppe *et al.* (2005) evaluated the relative bioavailability of elemental iron powders in humans, using a further developed serum iron method, standardised and validated with radioisotope absorption methods. The mean relative bioavailability (RBV) of electrolytic iron was 0.59 (59% compared to ferrous sulfate) (Hoppe *et al.*, 2005). Two recent efficacy trials in Kenya and South Africa also suggest that the bioavailability of electrolytic iron as iron fortificant is likely to be relatively low, since both studies showed no improvement in iron status among school children (Andang'o *et al.*, 2006; Van Stuijvenberg *et al.*, 2006). There is, therefore, a need to examine and determine the bioavailability of other alternative iron compounds that could be used in the South African National Food Fortification Programme. Ferrous fumarate and NaFeEDTA are both good alternative iron compounds that could possibly replace electrolytic iron as iron fortificant. These compounds will be discussed in more detail in Chapter 2 of this thesis.

**AIM**

The main aim of this study was to provide information about the bioavailability of ferrous fumarate and NaFeEDTA from maize meal porridge in young children, which would assist in selecting a bioavailable alternative to electrolytic iron in the South African National Food Fortification Programme.

**STRUCTURE OF THESIS**

This thesis is presented in article format. Following this preface, Chapter 1 consists of an introductory chapter. Chapter 2 consists of a review article on iron fortification of cereal food staples that serves as background to the main study (submitted for publication in the South African Journal of Clinical Nutrition). The objective of Chapter 2 is to review iron fortification of cereal food staples by discussing factors that influence iron absorption and bioavailability, as well as the current information about potential iron compounds for iron fortification with a specific focus on the South African situation.

In Chapter 3, the bioavailability of ferrous fumarate and NaFeEDTA from a maize porridge meal is investigated in children with low iron status (submitted for publication in the American Journal of Clinical Nutrition). In Chapter 4, a general discussion and summary of the results are provided, conclusions are drawn and recommendations made. The references of Chapters 2 and 3 are provided at the end of each chapter according to the authors' instructions of the specific journal to which the manuscript was submitted.

**CO-RESEARCHERS' CONTRIBUTIONS**

The experimental study reported in this thesis was planned and executed by a team of researchers. The contribution of each of the researchers is given in Table 1. Also included in this section is a statement from the co-authors confirming their individual roles in each study and giving their permission that the article may form part of this thesis.

The following is a statement from the co-authors confirming their individual roles in the experimental study and giving their permission that the article "Bioavailability of ferrous fumarate and NaFeEDTA from a maize porridge meal in children with low iron status" may form part of this thesis.

I declare that I have approved the above-mentioned article, that my role in the study, as indicated in Table 1, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the PhD thesis of Ms Z White.

Prof JC Jerling

Dr. Du Toit Loots

Steven a. Abrams, M.D.

Prof SA Abrams

Dr. DC Hilmers

Dr. Z Chen

Dr. M van Lieshout.

**Table 1** **List of co-researchers and their function in this study: Bioavailability of ferrous fumarate and NaFeEDTA from a maize porridge meal in children with low iron status.**

| Name | Role in the study |
|---|---|
| Ms. Z White M.Sc (Nutritionist) | All aspects considering the design, planning, execution and documentation of the study. Main author of the paper. |
| Prof. JC Jerling Ph.D (Nutritionist) | Study leader. All aspects considering the design, planning, approval of final protocol, funding, execution and documentation of the study. Statistical analysis. Critically revised paper. |
| Dr. Du Toit Loots Ph.D (Biochemist) | All aspects considering the design, planning and execution of the study. Preparation of stable isotopes. Critically revised paper. |
| Prof SA Abrams (Paediatrician) | Design and planning of study protocol. Critically revised paper. |
| Dr. DC Hilmers (Medical doctor) | Preparation of stable isotopes, execution of the study. Critically revised paper. |
| Dr. Z Chen | Laboratory analyses. Critically revised paper. |
| Dr. M van Lieshout Ph.D (Nutritionist) | Design and planning of study. Critically revised paper. |

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# CHAPTER 1

# INTRODUCTION

# 1. INTRODUCTION

## 1.1 Iron deficiency

Iron deficiency is the most common and widespread nutritional disorder in the world and is a public health problem in both industrialized and nonindustrialized countries (WHO/FAO, 2006). The lower levels of anaemia found in developed countries are attributed to higher levels of heme-iron intake and the fortification of staple cereals and other foods such as breakfast cereals (Nalubola & Nestel, 2000). In developing countries such as South Africa, diets are low in heme-iron and low in variety, consisting mainly of cereal products that lack sufficient iron content (quantity) or bioavailability (quality), thereby contributing to the low intake of dietary iron (Labadarios, 2000).

Iron deficiency (ID) is also caused by factors that increase the need for iron such as periods of rapid growth (for example, early childhood, adolescence, pregnancy), during blood loss (menstruation, childbirth), chronic losses from parasite infections (hookworms, schistosomiasis, whipworm) and malaria. Malaria, especially *Plasmodium. falciparum*, causes anaemia, but not ID, since the iron stays in the body, while some helminths such as hookworm and schistosomes cause blood and iron loss, and therefore ID. In severe cases this becomes iron deficiency anaemia (IDA). Anaemia can, therefore, be caused by iron deficiency or other factors (Nestel & Nalubola, 2000; Muller & Krawinkel, 2005).

Iron is present in all cells in the human body and it has several vital functions (FAO/WHO, 2002). The movement of oxygen from the environment to the tissues is one of the key functions of iron. Oxygen is bound to an iron-containing porphyrin ring, either as part of the prostetic group of haemoglobin within erythrocytes or as part of myoglobin as the facilitator of oxygen diffusion in tissues. Cytochromes contain heme as the active site with the iron-containing porphyrin ring, which act as electron carriers (Institute of Medicine, 2002). Adequate iron is important for the purpose of maintaining these vital functions in the body (CDC, 1998).

Iron deficiency is often portrayed as a progressive condition that begins with a normal body iron store that becomes subnormal or depleted because of low dietary iron intake, inadequate intestinal iron absorption or increased losses. As this process continues, synthesis of iron-containing proteins, such as haemoglobin, becomes compromised. Finally, when haemoglobin concentration falls below a specified cut-off value, the iron deficiency has progressed to iron deficiency anaemia (Haas & Brownlie IV, 2001).

Iron deficiency anaemia in early life is related to altered behavioural and neural development (Beard, 2003). When IDA ensues during the first 2 years of life, it is associated with delayed psychomotor development (cognitive skills: language acquisition, abstract thinking; and motor abilities: coordination, body balance, walking) as well as changes in behaviour. These cognitive deficits have been shown to persist at 5 to 6 and at 10 years of age, resulting in lower intellectual quotient (IQ), lower school achievement and poorer fine-hand movements (Walter, 2003).

In adolescents both ID and anaemia have been associated with poor concentration and cognitive performance, reduced appetite and reduced growth (Nestel & Nalubola, 2000). Physical working capacity is one of several areas of human performance that is impaired by iron deficiency, as has been reported widely. IDA reduces work capacity in adults by impairing aerobic capacity, endurance capacity (animal studies) and energetic efficiency, and possibly by decreasing voluntary activity and work productivity (Haas & Brownlie IV, 2001).

### 1.2 Dietary iron requirements

Dietary iron requirements depend on basal iron losses, the amount needed for growth and development as well as menstrual losses, thus, varying by age and gender. Table 1.1 provides the total absolute iron requirements as determined by growth requirement, basal iron losses and menstrual losses (females only). It further provides dietary requirements calculated for four levels of dietary iron bioavailability (FAO/WHO, 2002).

Table 1.2 gives the Dietary Reference Intakes (DRIs) for iron. The term DRIs is a collective one, and refers to a set of at least four nutrient-based reference values, namely estimated average requirement (EAR), recommended dietary allowances (RDA), adequate intake (AI) and upper intake level (UL). The RDA is defined as the intake that meets the nutrient needs of almost all (97-98%) individuals in that gender group, at the given life-stage. An AI is used in cases in which the scientific evidence is inadequate to set an estimated EAR and to have an RDA calculated. The tolerable upper intake level (UL) is defined as the maximum nutrient intake by an individual, which is unlikely to pose risks of adverse health effects in almost all (97-98%) individuals in a specific group (Institute of Medicine, 2002).

**Table 1.1** **Total absolute requirements and recommended intakes for iron based on varying dietary iron bioavailabilities** (FAO/WHO, 2002)

| Group | Age (years) | Body weight (kg) | Total absolute requirement[a] | | Recommended intake (mg/day) % Dietary iron bioavailability | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Median (mg/d) | 95th percentile (mg/d) | 15 | 12 | 10 | 5 |
| Children | 0.5-1 | 9 | 0.72 | 0.93 | 6.2[c] | 7.7[c] | 9.3[c] | 18.6[c] |
| | 1-3 | 13.3 | 0.46 | 0.58 | 3.9 | 4.8 | 5.8 | 11.6 |
| | 4-6 | 19.2 | 0.50 | 0.63 | 4.2 | 5.3 | 6.3 | 12.6 |
| | 7-10 | 28.1 | 0.71 | 0.89 | 5.9 | 7.4 | 8.9 | 17.8 |
| Males | 11-14 | 45 | 1.17 | 1.46 | 9.7 | 12.2 | 14.6 | 29.2 |
| | 15-17 | 64.4 | 1.50 | 1.88 | 12.5 | 15.7 | 18.8 | 37.6 |
| | 18+ | 75 | 1.05 | 1.37 | 9.1 | 11.4 | 13.7 | 27.4 |
| Females | 11-14 | 46.1 | 1.20 | 1.40 | 9.3 | 11.7 | 14 | 28 |
| | 11-14[b] | 46.1 | 1.68 | 3.27 | 21.8 | 27.7 | 32.7 | 65.4 |
| | 15-17 | 56.4 | 1.62 | 3.10 | 20.7 | 25.8 | 31 | 62 |
| | 18+ | 62 | 1.46 | 2.94 | 19.6 | 24.5 | 29.4 | 58.8 |
| Post-menopausal | | 62. | 0.87 | 1.13 | 7.5 | 9.4 | 11.3 | 22.6 |
| Lactating | | 62 | 1.15 | 1.50 | 10 | 12.5 | 15 | 30 |

[a]Total absolute requirements = Requirement for growth + basal losses + menstrual losses (females only)

[b]Non-menstruating

[c]Bioavailability of dietary iron during this period varies greatly

**Table 1.2** **The USA Dietary Reference Intakes (DRIs) for iron** (Institute of Medicine, 2002)

| Gender | Age | EAR | RDA | AI | UL |
|---|---|---|---|---|---|
| | Years | mg/day | mg/day | mg/day | mg/day |
| Male & Female | 0-0.5 | - | - | 0.27 | 40 |
| | 0.5-1 | 6.9 | 11 | - | 40 |
| | 1-3 | 3.0 | 7.0 | - | 40 |
| | 4-8 | 4.1 | 10 | - | 40 |
| Male | 9-13 | 5.9 | 11 | - | 40 |
| | 14-18 | 7.7 | 8.0 | - | 45 |
| | >19 | 6.0 | 8.0 | - | 45 |
| Female | 9-13 | 5.7 | 8.0 | - | 40 |
| | 14-18 | 7.9 | 15 | - | 45 |
| | 19-50 | 8.1 | 18 | - | 45 |
| | >51 | 5.0 | 8.0 | - | 45 |
| Pregnancy | ≤18 | 23 | 27 | - | 45 |
| | 19-50 | 22 | 27 | - | 45 |
| Lactation | ≤18 | 7.0 | 10 | - | 45 |
| | 19-50 | 6.5 | 9.0 | - | 45 |

EAR: Estimated average requirement; RDA: Recommended dietary allowances; AI: Adequate intake; UL: Upper intake level

The availability of dietary iron is determined by its chemical form (such as heme versus nonheme) and the presence of enhancers and inhibitors in the meal. Enhancers of nonheme iron absorption include animal tissue, ascorbic acid (vitamin C), organic acids such as citric and lactic, fermented soy products and cysteine-containing peptides. Nonheme iron absorption can be inhibited, on the other hand, by inhibitors like phytate, plyphenols, calcium, avidin (eggs), oxalic acid (for example, in spinach) soy protein, phosphates and other inorganic elements (such as Cu, Mn) (Heath & Fairweather-Tait, 2002).

### 1.3 Methods to control iron deficiency

Iron deficiency and anaemia can be corrected and prevented by increasing the dietary intake of iron and by reducing the underlying factors that prevent adequate iron absorption or increase iron losses. Interventions to prevent the loss of iron and to increase the supply of iron in populations whose diets have low amounts of readily absorbable iron include dietary modification, public health measures such as malaria control and deworming, supplementation and food fortification (Nestel & Nalubola, 2000). The fortification of food staples with iron, as a method to control iron deficiency, will be reviewed in more detail in Chapter 3.

### 1.4 The South African situation

In 1996 the South African Vitamin A Consultative group (SAVACG) published their findings of a nationwide survey done in 1994 on the anthropometric, vitamin A, iron and immunisation coverage status of children aged 6-71 months in South Africa. The data revealed a high prevalence of vitamin A deficiency and anaemia among South African children. One in five children in the country is anaemic (Hb≤11; Ferritin<12), one in fifteen is moderately anaemic (Hb 7≤10; Ferritin<10) and one in five hundred is severely anaemic (Hb<7; Ferritin<10). In terms of iron status, 10% of children were iron depleted or deficient (Hb≤11; Ferritin<12) and 5% had iron deficiency anaemia (Hb<11; Ferritin<12) (SAVACG, 1995).

In 1999, the first National Food Consumption Survey (NFCS) was undertaken in South Africa, with the primary objectives of determining usual food consumption and assessing the usual nutrient intake of children aged 1-9 years in South Africa. Nutrient intake findings indicated that for South African children as a whole, the dietary intake of iron was less than 67% of the RDA. Between 58% and 79% of children had dietary iron intakes of less than 67% of the RDA, and 41% to 63% of children had an iron intake of less than 50% of the RDA (Labadarios, *et al.*, 2000).

South Africa's current efforts to control micronutrient malnutrition were initiated by the findings of high prevalence of vitamin A deficiency and anaemia in children as published in the SAVACG report (SAVACG, 1995). The Department of Health established the Integrated Nutrition Programme, which aims to ensure optimal nutrition for all South Africans. Among other strategies, such as tackling the high prevalence of parasitic infestations in some areas, the government proposed a 3-way food-based approach to reducing malnutrition (Kloka, 2003).

Food fortification was chosen as part of South Africa's 3-way-food-based approach to combat micronutrient malnutrition in South Africa. The other two approaches include a micronutrient supplementation programme for women and children, and an educational programme to promote better dietary habits, including breast-feeding initiatives, school feeding programmes and campaigns to encourage people to grow their own vegetables and fruits to improve household food security as well as increasing intakes of micronutrient-rich foods (Kloka, 2003).

South Africa's National Food Fortification Programme (NFFP) is the result of a long and intensive process of stakeholder consultation and preparatory studies. The South African fortification task team, hosted by government, comprised stakeholders from the food industry, consumer organisations, professional food and nutrition associations, academic bodies, the Department of Agriculture, the Department of Trade and Industry and UNICEF. The Micronutrient Initiative also provided technical support. This task group implemented key activities in the development of the NFFP, such as the NFCS, industry situation analyses, a position paper on iron fortificants, stability tests and organoleptic evaluations, advocacy and communication campaigns, fortification standards, regulations and monitoring plans, the development of a database of small-scale millers and the training and capacity building of small-scale millers and Environmental Health Practitioners (De Hoop & Matji, 2002).

The official launch of South Africa's NFFP took place on April 1st, 2003. Regulations pertaining to the mandatory fortification of all maize meal and wheat flour were published in the Government Gazette on 7 April 2003, under Act no.54 of 1972 Foodstuffs, Cosmetics and Disinfectants. These regulations became legally applicable and implementable on 7 October 2003 (DOH, 2003).

Fortification requires the identification of commonly eaten foods that can act as vehicles for one or more micronutrients, and lends itself to centralized processing on an economical scale (UNICEF *et al.*, 1998). In the South African mandatory fortification regulations, maize (sifted, special, super) and white and brown wheat flour were chosen as the vehicles for fortification (DOH, 2003). The NFCS provided information regarding nutrient intake as well as the identification of a suitable food fortification vehicle(s) that is consumed frequently and in sufficient quantities by the target population. The survey found that at the national level, the five most commonly eaten foods included maize, white sugar, tea, whole milk and brown bread. Many South

African children, particularly the poor, rely almost exclusively on maize porridge for their nutrition. The NFCS also indicated a median consumption of 500g/day maize porridge for children aged 7-9 years old and 420 g/day for those aged 1-3 years old. The medium consumption of bread per day is 101g for brown and 96g for white bread in children (Labadarios *et al.*, 2000).

According to the mandatory fortification regulations, maize and wheat flour should be fortified with 6 vitamins (Vitamin A, thiamine, riboflavin, nicotinamide, pyridoxine and folic acid) and 2 minerals (iron and zinc). The regulations specify the amount of fortificant that needs to be put in these products, and they further stipulate the requirements for compliance and monitoring to ensure that fortification is done according to the regulations (DOH, 2003).

Elemental iron, specifically electrolytic iron is used as the iron fortificant in the South African food fortification programme. Table 1.3 gives the specified requirements for electrolytic iron in wheat flour and maize meal. The iron should be elemental iron in which more than 95% passes through a mesh (<45 microns particle size) made by an electrolytic process. Table 1.4 provides the iron composition of the fortified foodstuffs (DOH, 2003).

**Table 1.3 Iron requirement for fortification of food vehicles** (DOH, 2003)

| Food vehicle | RDA | Iron requirement | | |
|---|---|---|---|---|
| | | Per 200g Nutritional goal | | Per 1 kg Required Addition (mg) |
| | | %RDA | Amount (mg) | |
| Wheat flour | 14 | 50% | 7.0 | 35.0 |
| Maize meal | 14 | 50% | 7.0 | 35.0 |
| Unsifted maize meal* | 14 | 25% | 3.5 | 17.5 |

* Manufactures, importers and suppliers of un-sifted maize meal may apply to the Director-General of the Department of Health for special permission to use a fortificant mix with a reduced level of electrolytic iron.

**Table 1.4 Iron content of fortified foodstuffs** (DOH, 2003)

| | Composition per 1kg flour/bread | | | | |
|---|---|---|---|---|---|
| | **Fortification** | **Natural** | **Total** | **Tolerance** | **Netto** |
| White bread flour | 35.0 | 13.5 | 48.5 | ±10% | 43.7 |
| Brown bread flour | 30.8 | 22.5 | 53.3 | ±10% | 48.0 |
| White bread | 23.3 | 12.5 | 35.8 | ±10% | 32.3 |
| Brown bread | 20.5 | 18.0 | 38.5 | ±10% | 34.7 |
| Super maize meal | 35.0 | 6.5 | 41.5 | ±10% | 37.4 |
| Special maize meal | 35.0 | 9.6 | 44.6 | ±10% | 40.1 |
| Sifted maize meal | 35.0 | 14.2 | 49.2 | ±10% | 44.3 |
| Unsifted maize meal | 35.0 | 21.0 | 56.0 | ±10% | 50.4* |

* Where special permission was granted, a lower netto iron content of 34.65 mg/kg shall be applicable

## 1.5 Measurement of iron bioavailability

The bioavailability of iron is defined as the degree in which iron is absorbed in the gastrointestinal tract and utilized for normal metabolic functions, for example, incorporation into haemoglobin. It is expressed as a percentage of the total amount of the nutrient (Nestel & Nalubola, 2000). Stable isotopes provide the only direct way to measure iron uptake and bioavailability and are regarded as the "gold standard" for iron studies in humans and other studies of nutrient bioavailability. Stable isotopes are completely safe and non-invasive and can be used in free-living humans, since they emit no externally measurable radiation (Iyengar, 2002). The bioavailability of iron will be reviewed in more detail in Chapter 3.

Elements such as iron can exist in both stable and unstable (radioactive) forms. Stable iron atoms have the same atomic number (protons) as the element iron, but differ in atomic weight (neutrons). Due to the identical number of protons, these isotopes occupy the same (*isos*) position (*topes*) in the periodic table of elements (Koletzko *et al.*, 1998).

Iron is the only trace element of which a direct measure of bioavailability has been developed (Fairweather-Tait & Dainty, 2002). Three of the four stable isotopes of iron ($^{54}Fe$, $^{57}Fe$ and $^{58}Fe$) have a natural abundance that is low enough for iron bioavailability studies (5.8%, 2.2% and 0.3%, respectively) (Heath & Fairweather-Tait, 2002). The lowest-abundance isotopes, $^{58}Fe$ and $^{57}Fe$, are most commonly used in human nutrition research (Abrams, 1999).

In absorption studies, a known amount of isotope is consumed at the same time as a test meal (that is, the meal is extrinsically labelled with iron), and the isotope is assumed to be absorbed and metabolised by the body in the same way as the food iron (Heath & Fairweather-Tait, 2002). The ratio of the administered isotope ($^{58}Fe$ or $^{57}Fe$) is determined relative to $^{56}Fe$ in the sample of blood (Abrams, 1999). Since the majority of newly absorbed iron is incorporated into reticulocytes (immature red blood cells), the proportion of an oral dose of isotopically labelled iron that is found in blood haemoglobin can be used to determine bioavailability. Isotopic enrichment of a blood sample taken fourteen days after the oral dose is quantified by mass spectrometry and blood volume estimated from the height and weight of the subject (Fairweather-Tait & Dainty, 2002).

## 1.6 Conclusion

Iron fortification of food is generally considered to be the best long-term strategy to increase iron intake and has been reported to contribute to iron intake among those consuming fortified foods in developed countries, where it has been practised for many years (Nestel & Nalubola, 2002). The use of stable isotope techniques provides the most direct way to measure/evaluate the bioavailability of a potential iron fortificant in the context of the meal in which it is to be consumed.

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## CHAPTER 2

# IRON FORTIFICATION OF CEREAL FOOD STAPLES: A REVIEW

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## INTRODUCTION

The term "food fortification" refers to the addition of one or more essential nutrients to a food, regardless of whether it occurs naturally in the food. The purpose of micronutrient fortification is to correct a recognised population-wide micronutrient deficiency or to add micronutrients lost in processing back to their original levels (known as restoration) or even higher.[1]

Because iron deficiency (ID) and iron deficiency anaemia (IDA) affect all age groups and all strata of society, including many not served by the public health or welfare systems, iron fortification of food has distinct advantages over the other interventions, such as dietary modification, supplementation and public health measures, such as malaria control and deworming.[1] According to Baltussen *et al.*,[2] iron fortification is economically more attractive than iron supplementation, because it appears to be more cost effective, regardless of the geographic coverage of fortification.

Iron fortification is one of the least expensive and potentially most effective strategies to supply micronutrients to at-risk populations, because of the low cost of iron fortification of flour and the large potential health gains in populations in which ID and IDA are prevalent.[1,3] Fortification is a feasible approach to prevent iron deficiency on a population-wide scale for a number of reasons: technical feasibility has been well established; the cost of fortification is relatively low; there is evidence from several parts of the world that iron fortification is effective; once it has been established, iron fortification does not require any special investment in promotion or education; and finally, flour fortification can deliver other vitamins and minerals.[4] Staple food fortification, however, does have its limitations. Apart from the iron quality to assure good absorption, the distribution of the fortified food and the amount consumed could have an impact on iron fortification. According to Dary,[5] fortification of staple foods should be complemented with the implementation of other interventions in order to really overcome iron deficiency.

A clear understanding of factors that influence iron absorption is critical to the design of effective fortification strategies.[6] This paper will review iron fortification of cereal food staples by discussing iron absorption and bioavailability, as well as by giving a brief overview of the current information about potential iron compounds for fortification.

## IRON ABSORPTION AND BIOAVAILABILITY

Regulation of iron balance occurs mainly in the gastrointestinal tract through absorption.[7] Nonheme iron is absorbed early in digestion, mainly in the duodenum by the absorptive epithelial cells of the intestine (enterocytes) through the apical (luminal) membrane of the enterocyte.[8,9]

The first step in iron absorption is the transfer across the apical membrane into the enterocyte.[9] Apical uptake of iron is mediated by the divalent metal ion transporter DMT1. The primary location of DMT1 in the gut is on the brush-border membrane of mature villous enterocytes of the proximal duodenum, where the expression of DMT1 is tightly regulated by body iron status.[10]

Nonheme iron is present in the diet either in the reduced ferrous ($Fe^{2+}$) form or in the oxidized ferric ($Fe^{3+}$) form.[8] Since most dietary nonheme iron is in the ferric ($Fe^{3+}$) form, it must first be reduced to ferrous ($Fe^{2+}$) iron.[9] $Fe^{3+}$ is reduced to $Fe^{2+}$ by ascorbic acid and apical membrane ferrireductase that includes duodenal cytochrome B (DcytB). The acid microclimate at the brush border provides an $H^+$ electrochemical potential gradient to drive transport of $Fe^{2+}$ via the divalent metal-ion transporter DMT1 into the enterocyte.[10]

Once inside the enterocyte, iron has two possible fates. Some remains stored within the cell: this iron is ultimately lost from the body at the end of the enterocyte lifespan. The remainder is transferred across the basolateral surface.[9] Basolateral export of $Fe^{2+}$ may be mediated by IREG1 (ferroportin1) in association with hephaestin. It is thought that the ferroxidase hephaestin is an electron acceptor associated with the basolateral export of $Fe^{2+}$ by IREG1/ferroportin1 before handing $Fe^{3+}$ off to transferrin.[10]

Nonheme iron absorption is conditioned by a multitude of factors, such as the chemical form in which it is present in the food (in the ferrous state it is absorbed much better than in the ferric state), organic acids, gastric acid secretion, the amount and kind of iron in the diet, amount of iron in the body, rate of red blood cell production and the presence of certain enhancers and inhibitors.[7,10,11]

The amount of iron available for absorption in the gut is also dependent on its solubility in gastric juice, which in turn is dependent on the chemical and physical characteristics of the compound (size, shape and surface area of particles).[12] Gastric acid secretion may be more critical for the absorption of some forms of fortification iron.[6] Non-water-

soluble compounds (such as elemental iron) are poorly soluble in gastric secretions, while ferrous fumarate is soluble in dilute acid.

*Helicobacter pylori* (*H.pylori*) infection affects gastric acid secretion. *H.pylori* infections are caused by a bacterium isolated from the gastric mucosa, which results in low gastric acid secretion. In developing countries more than 50% of children are infected by the age of 10 years,[13] and the hypothesised resulting hypochlorhydria may compromise nonheme iron absorption.

Results from the study by Sarker *et al.*,[14] however, do not support the hypothesis that *H.pylori* infection influences iron absorption from water-soluble or non-water-soluble iron. This study measured iron absorption from ferrous sulfate and ferrous fumarate in 2-5 year old children with and without *H.pylori infection*. They found that reduced gastric secretion associated with *H.pylori* infection did not significantly influence iron absorption from the two iron compounds. More research is needed, however, to determine the effect of *H.pylori* infection on iron absorption from other iron compounds.

If the incidence of hypochlorhydria is shown to be high, considerations may need to be given to the selection of iron fortificants that are less dependent on gastric acid secretion.[6]

Most nonheme iron $Fe^{2+}$ and $Fe^{3+}$ in food are complexed with organic acids (such as citrate) or peptides (such as ferritin and albumin), and these are not limited to $Fe^{2+}$ salts. Bioavailability of nonheme iron may, therefore, be determined in large part by the solubility of such complexes, and the affinity with which they bind iron.[10]

The percentage of iron absorbed (that is, iron bioavailability) can vary from <1% to >50%.[7] This review focuses on the bioavailability of nonheme iron compounds added to fortified cereal food staples as measured by isotope studies.

## SELECTION OF IRON COMPOUND FOR FOOD FORTIFICATION

The relative bioavailability of iron compounds is determined by their solubility in the stomach's gastric juice.[15] To be effective, an iron compound must be soluble in human gastric juice. Ferrous sulfate is highly soluble in water and gastric juice, and it is the cheapest and most widely used iron salt for food fortification. The relative bioavailability of other iron compounds is characterized by comparing their bioavailabilities with ferrous sulfate (relative bioavailability of ferrous sulfate = 100%).[16]

The choice of iron compound should be based on bioavailability of the compound and sensory evaluations of the fortified food. Local conditions, such as temperature, humidity, packaging, storage time, food preparation and consumption patterns should be considered in addition to cost.[17] Table 1 shows the characteristics of commonly used iron fortificants.

### ❑ Conventional iron compounds

Iron compounds can be classified into the following categories according to their solubility in the stomach's gastric juice:[18]

- **Freely water soluble: ferrous sulfate**

Freely water-soluble compounds have the highest relative bioavailability of the conventional iron compounds, and they should be the first choice for food fortification, provided that they are accepted organoleptically. Ferrous sulfate is the only widely used water-soluble compound that is commonly added to food, but it can only be added to a small number of food vehicles in view of its high potential for adverse organoleptic changes, that is, colour and fat oxidation.[22] It is very difficult to add ferrous sulfate to foods without changing organoleptic quality of the food vehicle and threatening consumer acceptance. It is usually used only in infant formulas, short shelf-life bread and some pastas.[15]

Because of its high bioavailability and low cost, FCC (Food Chemicals Codex, Vol.IV) grade dried ferrous sulfate is often the best iron source, and this can be used in bakery flour, semolina and other types of low extraction wheat flours, which are normally used within one to two months after production.[19] Ferrous sulfate is more likely to cause storage and sensory problems in high extraction "brown" flours, or in foods with extraction rates above 82%, than in low extraction (72-78%) "white" flours, because of their higher unsaturated fat content.[19]

The use of ferrous sulfate may not be appropriate in products stored for extended periods, due to its promotion of oxidative rancidity of native or added fats, which reduces acceptable shelf life. It can also produce changes in colour and flavour over time, which would reduce consumer acceptance. Ferrous sulfate is not recommended for flour used in mixes with added fat or home-use all-purpose flour requiring an extended shelf life of over three months.[19]

**Table 1 Characteristics of commonly used iron fortificants**[12,17]

| Fortificant | Approximate iron content (%) | Average relative bioavailability | Potential for adverse organoleptic changes |
|---|---|---|---|
| Freely water soluble | | | |
| Dried ferrous sulfate | 32 | 100 | High* |
| Poorly water soluble/ soluble in dilute acid | | | |
| Ferrous fumarate | 33 | 100 | Low |
| Ferric saccharate | | 74 | |
| Water insoluble/ poorly soluble in dilute acid | | | |
| Ferric pyrophosphate | 25 | 21-75 | |
| Ferric orthophosphate | 28 | 25-32 | |
| Elemental iron: | | | |
| Electrolytic iron | 97 | 50 -100 | Negligible |
| H-reduced iron | 97 | 13-148 | Negligible |
| CO-reduced (sponge) iron | 97 | No data | Negligible |
| Carbonyl | 99 | 5-20 | |
| Chelates | | | |
| Sodium iron EDTA | 13 | 150-300 | Medium to low |

*Ferrous sulfate promotes the oxidation of fat found in flour during storage and results in rancidity

- **Poorly water soluble OR soluble in dilute acid: ferrous fumarate, ferrous succinate and ferric saccharate**

If the water-soluble compounds cause unacceptable sensory changes to the food vehicle, the next step is to evaluate compounds that are poorly soluble in water, but soluble in dilute acid. These compounds cause less organoleptic changes than water-soluble compounds, but they have a similar or slightly lower relative bioavailability in healthy adults. As a result of these organoleptic difficulties of water-soluble compounds, the poorly water-soluble compounds, such as ferrous fumarate are

increasingly used in fortification. Although there is a slight risk of colour change, it can be used in infant cereals, maize meal and chocolate drink powders.[15] Unpublished data from Alvarado and De Pereda have respectively shown that ferrous fumarate does not cause undesirable sensorial changes in wheat flour of up to 60 mg/kg and nixtamalised corn flour of up to 30 mg Fe/kg.[20] Only ferrous fumarate and ferric saccharate are widely used as iron fortificants.[18]

- **Water insoluble OR poorly soluble in dilute acid: elemental iron powders (electrolytic, carbonyl and reduced), phosphate iron compounds (ferric pyrophosphate & ferric orthophosphate)**

Compounds that are insoluble in water and poorly soluble in dilute acid, such as iron phosphates and elemental iron powders, should be the last choices for food fortification, because these compounds are the least well absorbed of the iron fortificants.[17] The iron absorption of these compounds are difficult to predict, because they dissolve slowly and incompletely in gastric juice during digestion and depend on physical characteristics (size, shape and surface area of particles) of the compound as well as the consumer's gastric acid secretion and the composition of the meal.[17] Some compounds from this group can be useful fortificants, whereas it may never be able to ensure adequate absorption with others.[18]

Elemental iron powders are the most common iron fortificants used worldwide, because they cause the fewest organoleptic and stability problems in food products and they are relatively inexpensive.[21] A 2002 review by an expert panel concluded that electrolytic iron powder (<45 $\mu$m, 325 mesh, with a dendritic structure similar to Glidden A131) is the only elemental iron powder that has been demonstrated to be a useful iron fortificant,[22] while it is "also the only elemental iron compound for which there is evidence of absorption by humans". However, even under optimal dietary conditions, this iron is absorbed only one-half as much as ferrous sulfate.[23]

Electrolytic iron, fortified into wheat-based snacks, significantly improved iron status in Thai women with low iron stores (12mg Fe/d for 6d/wk for 35 wk).[24] More recent efficacy trials on electrolytic iron did not support the use of electrolytic iron as an iron fortificant. The efficacy of electrolytic iron as a fortificant was evaluated in two trials with whole maize and brown bread as the fortification vehicles.[25,26] There was no evidence that fortification of maize meal with electrolytic iron (56mg/kg) consumed for 20 weeks (5d/wk) resulted in improved iron status among school children.[25] Van Stuijvenberg *et*

*al.*[26] also found no significant treatment effects in children consuming bread fortified with electrolytic iron (35mg/kg for 4.5mo and 70mg/kg for 3mo).

Due to the limited information available, no decision about the usefulness of hydrogen (H)-reduced, carbon monoxide (CO)-reduced, atomised or carbonyl iron powders was made in cases in which elemental iron was evaluated for cereal flour fortification.[22]

- **Novel iron compounds**

Other alternatives to be considered for iron fortification are sodium iron ethylenediaminetetraacetic acid (NaFeEDTA) and ferrous bisglycinate.

- **Sodium iron ethylenediaminetetraacetic acid (NaFeEDTA)**

NaFeEDTA, also called iron EDTA, has a potential role as an iron fortificant for food fortification. The Joint FAO/WHO Expert Committee on Food Additives approved NaFeEDTA at a maximum daily intake of 0.2mg iron/kg body weight for food fortification in supervised programmes in areas with a high prevalence of iron deficiency.[27] The major advantage of NaFeEDTA over other fortification compounds is that it prevents iron binding to phytate, a potential inhibitor of iron absorption.[18]

NaFeEDTA, which is pale yellow in colour, causes fewer organoleptic problems than other water-soluble iron compounds, while it also does not promote fat oxidation in stored wheat flour and is stable during processing and storage.[28,29]

Iron from NaFeEDTA forms a common pool with nonheme iron,[30,31] and improves the absorption of nonheme iron sources.[30,32] Absorption of iron from NaFeEDTA is found to be 2-4 times higher from infant cereals or bread rolls than when fortified with iron compounds, such as ferrous sulfate or ferrous fumarate.[22] NaFeEDTA consistently indicates 2-3 times better absorption than ferrous sulfate. This better absorption is, however, limited to high phytate meals. When there is no phytate present, NaFeEDTA is similar to or displays even less absorption than ferrous sulfate.[15] Although NaFeEDTA enhances iron absorption from foods containing phytate, the study by Hurrell *et al.*[29] suggests that iron absorption is also influenced by the amount of phytate present in the food matrix. Iron absorption from NaFeEDTA in a high-extraction-wheat bread roll (3.91%) was much lower than a low-extraction-wheat roll (11.5%) in which phytate was degraded to zero.[29]

NaFeEDTA, however, has disadvantages: it causes undesirable changes in the sensorial properties of wheat and nixtamalized (lime-treated) maize flours, since NaFeEDTA or EDTA alone affect the dough viscosity of wheat flour and specific volume of bread.[20] According to Kuyper, consumer tests carried out on maize meal and wheat flour fortified with NaFeEDTA (18 mg Fe/kg) also revealed colour and taste differences in cooked porridge and bread.[28] However, according to Rodenstein, maize meal fortified with NaFeEDTA (20 mg Fe/kg) is successfully marketed in Kenya. [28]

Andang'o *et al.*[25] evaluated the efficacy of two levels of NaFeEDTA as iron fortificant in whole maize meal on iron status of Kenyan school children. Low-NaFeEDTA (28mg/kg) and high-NaFeEDTA (56mg/kg) resulted in marginal and modest improvements of iron status respectively, when consumed for 20 weeks (5d/wk).[25] Wheat flour fortified with NaFeEDTA (20mg/kg) resulted in significantly higher efficacy in treating anaemia and improving iron status in anaemic children compared to ferrous sulfate (30mg/kg) and electrolytic iron (60mg/kg) after 6 months.[33]

$Na_2EDTA$ can be added to foods in cases in which NaFeEDTA causes unacceptable sensory changes or in cases in which it is considered too expensive.[29] The enhancing effect of $Na_2EDTA$ on iron absorption has been shown for freely water-soluble compounds, such as ferrous sulfate. The addition of $Na_2EDTA$ to poorly water-soluble compounds, such as ferrous fumarate, has been found to enhance iron absorption from maize-masa tortillas.[34] Other studies, however, showed no enhancing effect of $Na_2EDTA$ to iron absorption from ferrous fumarate in maize tortillas and black bean paste[35] or from a wheat-based infant cereal.[36] The effect of $Na_2EDTA$ on the bioavailability of ferrous fumarate from other maize based meals, such as maize porridge, has not been examined. Further research on the enhancement effect of $Na_2EDTA$ is necessary.

- **Ferrous bisglycinate**

Ferrous bisglycinate has been developed commercially, but a completely independent evaluation of its bioavailability has not been possible.[17] The efficacy of ferrous bisglycinate as a fortificant in brown bread was evaluated by Van Stuijvenberg *et al.*,[26] by comparing it to electrolytic iron in a randomised controlled trial. They observed significant treatment effects for haemoglobin, serum iron and transferrin saturation, but not for serum ferritin. Overall, ferrous bisglycinate performed better than electrolytic

iron as iron fortificant in brown bread in a group of iron-deficient school children as tested over a period of 7.5 months (35mg/kg for 4.5mo and 70mg/kg for 3mo).[26]

Ferrous bisglycinate does not cause undesirable sensorial changes in wheat flour at concentrations of up to 22.5 mg/kg.[20] However, ferrous bisglycinate caused rancidity and lowered the sensory quality and storage stability of maize in Bovell-Benjamin and Co-workers's[37] evaluation of the sensory quality and storage stability of whole maize fortified with different iron salts and iron chelates.

Ferrous bisglycinate has a bioavailability 2-4 times that of ferrous sulfate.[38,39,40] The high cost of ferrous bisglycinate is one of the main limitations that debars its widespread use. It is approximately 20 times more expensive than ferrous sulfate per unit of iron. According to Hertrampf and Olivares,[41] additional research, including well-designed efficacy studies, is necessary to establish the cost-effectiveness of using ferrous bisglycinate in foods that can be fortified with bioavailable iron salts, in addition to determining the appropriate fortification levels.

## OPTIMISING IRON BIOAVAILABILITY

Iron is the most difficult mineral to add to foods while ensuring adequate bioavailability by the body.[15] Iron is a reactive compound, whose level in foods is limited because iron causes negative changes in the original properties of the food that is being fortified.[5] Highly bioavailable iron compounds can cause organoleptic problems that raise issues of consumer acceptance, while on the other hand, iron compounds that do not cause organoleptic problems, are not as bioavailable and are poorly absorbed.[15] Different strategies are available for increasing the bioavailability of fortification iron from diets, and the key issues towards their application in cereal food staples will be discussed.

### ❑ Phytate removal or degradation

Phytate is a potent inhibitor of absorption for native and fortification iron in cereals and legume-based foods,[42] because of the poor solubility of its iron chelate at any pH level.[10]

*Myo*-Inositol-1,2,3,4,5,6-hexa*kis*phosphate (Ins P6), first known as "phytic acid," is the major form of phosphorus in seeds and other plant tissues, and functions in the storage and retrieval of phosphate, myo-inositol (Ins) and minerals during development and germination of these tissues. In addition, Ins P6 serves as a major metabolic pool in Ins

phosphate and pyrophosphate pathways involved in signal transduction and regulation. Ins P6 and its derivatives also function in RNA export, DNA repair, DNA recombination, in endocytosis and vesicular trafficking, and as an anti-oxidant.[43]

Effective enhancement of iron absorption requires near-complete degration or removal of phytate.[42] According to Nestel and Nalubola,[44] research suggests that the maximum iron absorption benefit, that is, a threefold to fivefold increase, can be achieved only by complete removal or degradation of phytate. However, this is not always practical. A nutritionally significant increase (about a twofold) in iron absorption can be obtained by decreasing the phytate content to a phytate:iron molar ratio of 0.4:1-1:1.[44] Complete dephytinization of cereal and legume-based complementary foods has been shown to lead to as much as a 12 fold increase in the percentage of iron absorption (0.99% to 11.54%), as found in a single-meal study in which the foods were reconstituted with water.[45]

There are two major food processing methods that can be used to decrease the inhibitory effect of phytate on iron absorption, namely phytate removal and enzymatic degradation. Milling of cereals can cause up to a 90% reduction of phytate, since it is removed in the bran together with most of the dietary fibre. Soaking and germination are traditional processes that activate the native phytases in the grains and seeds, which then degrade phytate by successive removal of the phosphate groups.[45] Ins P6 also accumulates during seed development and is broken down during germination,[46] resulting in a reduced phytate content and increased iron bioavailability.

Fermentation with food-grade bacteria similarly activates native phytases by reducing the pH level through the production of organic acids, thus optimising the conditions for the native phytases. The addition of exogenous/commercial phytase is probably the easiest way to degrade phytate to zero, by holding the cereal mixture in aqueous solution at the optimum condition for phytases activity. Exogenous phytases have not been extensively evaluated as a means of improving the absorption of fortification iron from human foods when they are added after processing.[45] Questions about technical feasibility and cost need to be resolved for food uses.[42]

- **Encapsulation of iron compounds**

Encapsulated iron compounds are in development and some are already commercially available and mainly used to fortify infant formula and cereals.[47] Encapsulation is a

process in which the iron compound is encapsulated with a continuous layer or layers of coating material that separates the iron compound from the food matrix.[42]

Iron encapsulation has the potential to help overcome several major challenges in iron fortification of foods. The main advantage of encapsulation is that it should allow the addition of iron compounds of high relative bioavailability into difficult food vehicles, such as cereal flours, without causing the customary colour and flavour changes, and possibly reduce interactions with food components that lower iron bioavailability. There is, however, no evidence that microencapsulation will enhance the absorption of fortification iron, and bioavailability can potentially be decreased by encapsulation. Several factors may influence bioavailability from encapsulated products, including capsule material, the ratio of capsule material to iron and the technology and process used for encapsulation. Further research on the bioavailability of encapsulated iron compounds and organoleptic testing is necessary before they can be used for large-scale fortification.[42,47]

- **Addition of ascorbic acid**

Ascorbic acid is the most efficient enhancer of nonheme iron absorption when its stability in the food vehicle is ensured.[48] The enhancing effect of ascorbic acid is related to its reducing power and chelating action. Ascorbic acid, either as derived from the diet or as derived from gastric or biliary secretions, can efficiently reduce ferric iron in a low-pH environment. Even at higher pH levels, ascorbate forms soluble $Fe^{2+}$ or $Fe^{3+}$ complexes that promote iron absorption.[10]

The magnitude of the effect of ascorbic acid depends on the amount of ascorbic acid added, the stability of ascorbic acid, the type and concentration of iron fortification and the amount of inhibitors present in the meal.[18,48]

Ascorbic acid is not easily formulated into many finished food products because of its sensitivity to heat, water and oxygen. Ascorbic acid is reasonably stable in air in the dry state, but oxidises rapidly in solution, and may also darken upon exposure to light, moisture and heat. The instability of ascorbic acid during storage, heat processing and cooking as well as the cost of ascorbic acid itself or the cost of effective packaging are major reasons why it is not used to enhance fortificant iron added to food staples, such as wheat flour and maize meal.[48]

## BIOAVAILABILITY OF DIFFERENT IRON COMPOUNDS IN CEREALS

Table 2 presents a summary of the bioavailability of different iron compounds in cereal foods as determined by isotopic studies. Table 3 shows the preferred and optional iron compounds normally used to fortify cereals, based on relative bioavailability and sensory evaluations of the fortified food, as summarised by Nestel and Nalubola.[12]

**Table 2 Bioavailability of different iron compounds in cereal foods**

| Food matrix | $FeSO_4$ | FeFum | Ferrous bisglycinate | NaFeEDTA | Reference |
|---|---|---|---|---|---|
| White wheat flour | 5.3 | | 10.8 | 14.9 | 45 |
| Wheat | 6.2 | | | 14.6 | 46 |
| Low-extraction wheat bread roll | 5.70 | | | 11.50 | 35 |
| High-extraction wheat bread roll | 0.99 | | | 3.91 | 35 |
| Wheat infant cereal | 2.2 | 2.06 | | 5.2 | 35 |
| Precooked maize meal | 4.7 | | 8.4 | 10.5 | 45 |
| Lime-treated maize meal | 5.5 | 5.5-6.2 | | 9.0 | 41 |
| Maize masa flour tortillas | | 0.87 | 1.27 | 5.30 | 40 |
| Maize meal | 4.0 | | | 8.2 | 37 |
| Whole-maize porridge | 1.3 | | 6.4 | | 47 |
| Maize porridge | 1.7 | | | 5.7 | 48 |

$FeSO_4$: Ferrous sulfate, FeFum: Ferrous fumarate; NaFeEDTA: Sodium iron EDTA

**Table 3 Potential use of different iron forms in the fortification of cereals**[12]

| Cereals | Extraction rate (%) | Preferred iron compound | Alternative iron compound |
|---|---|---|---|
| Whole wheat flour (atta) | 97 | Sodium iron EDTA | Electrolytic iron, ferrous fumarate |
| All-purpose flour* | 75 | Electrolytic iron | Ferrous fumarate |
| Bread flour | 75 | Dried ferrous sulfate | Electrolytic iron, ferrous fumarate |
| Semolina | 60-65 | Dried ferrous sulfate | Electrolytic iron, ferrous fumarate |
| Cake flour | 50-55 | Electrolytic iron | Ferrous fumarate |
| Pastry flour | 45 | Electrolytic iron | Ferrous fumarate |
| Corn flour/ nixtamilized corn flour/ maize meal | 75-95 | Electrolytic iron (low extraction); Sodium-iron EDTA (high extraction) | Ferrous fumarate (low extraction) |

*Because ferrous sulfate is appropriate only for flours that are used within 1-2 months of production, and because all-purpose flour for home use may not be used within this time, electrolytic rather than dried ferrous sulfate is listed as the preferred iron compound.

## CHOICE OF FORTIFICATION LEVEL

The level of iron fortificant should be based on information about dietary intakes of iron by the target population, their requirement for absorbed iron, the expected absorption of iron from the fortified food and the consumption pattern of the food. It might be necessary to add 2-3 times more iron in cases in which more insoluble compounds such as elemental iron powders or iron phosphates are used, in contrast with ferrous sulfate.[17]

The minimum addition level recommended to restore the iron present in the whole grain product is 25 ppm iron for white flour, using ferrous sulfate or ferrous fumarate. This would give an iron level in the enriched flour of about 35 ppm or equivalent to the original level found in whole wheat flour. Because of the lower bioavailability of elemental iron powders compared to soluble iron salts, the addition rate of an elemental iron powder should be twice that of iron from the iron salts. For example, 50 ppm iron should be added in the form of electrolytic iron in place of adding 25 ppm from ferrous sulfate.[19] Table 4 shows the upper-level sensory threshold (ppm) for iron fortificants added to wheat flour, as summarised by Nestel and Nalubola in a technical brief on iron compounds for fortification of staple foods.[12]

**Table 4 Upper-level sensory threshold (ppm) for iron fortificants added to wheat flour**[12]

| | Wheat flour stored up to 3 months | |
|---|---|---|
| | High temperature (30-40 °C), high relative humidity (70-80%) | Low-moderate temperature (20-30 °C), low relative humidity (<50%) |
| Ferrous sulfate | 30 | 40 |
| Ferrous fumarate | 60 | NA |
| Electrolytic iron | 66 | NA |
| H-reduced iron | 66 | 88 |
| CO-reduced iron | NA | NA |
| NaFeEDTA | 15 | NA |

NA = published data or study results not available

## CONCLUSION

Iron fortification of food is generally considered to be the best long-term strategy to increase iron intake.[12] The goal of iron fortification is to provide vulnerable populations with iron in a bioavailable form that meets their bodies' needs and benefits their growth, development and productivity.[3]

The least absorbable iron forms, such as water insoluble compounds such as elemental iron, are widely used in the fortification of cereals, because they do not cause any organoleptic problems.[15] Nonetheless, their bioavailability is questioned. Although ferrous sulfate has the highest relative bioavailability, its use in cereals stored for longer periods of time is not practical due to unwanted organoleptic changes. The use of ferrous fumarate in fortification is increasing, because it causes fewer sensory changes than ferrous sulfate and it has a good relative bioavailability. NaFeEDTA appears highly suitable as an iron fortificant for the fortification of cereal-based foods, especially those with high phytate concentrations. Iron absorption can also be optimised by several strategies, including removal of absorption inhibitors, addition of absorption enhancers or encapsulation of the iron.

The iron compound used to fortify a specific food should be the compound with the highest relative bioavailability that causes no adverse sensory changes.[18] Appropriate information about bioavailability, technological compatibility as well as cost effectiveness analyses is needed before a decision regarding the use of an iron compound in food fortification programmes can be made.

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## CHAPTER 3

# BIOAVAILABILITY OF FERROUS FUMARATE AND NAFEEDTA FROM A MAIZE PORRIDGE MEAL IN BLACK AFRICAN CHILDREN

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## ABSTRACT

**Background:** In sub-Saharan Africa, maize is the staple food for an estimated 360 million people. In South Africa it is mandatory to fortify maize meal with iron, as well as other nutrients. Elemental iron, specifically electrolytic iron, is currently the preferred choice but other compounds that might be more effective in alleviating iron deficiency are under consideration.

**Objective:** The aim was to provide information about the bioavailability of ferrous fumarate and NaFeEDTA from maize meal porridge in young black African children, which would assist in selecting a bioavailable alternative to elemental iron.

**Methodology:** Iron bioavailability from ferrous fumarate and NaFeEDTA was measured and compared to ferrous sulfate in 5-year-old South African children. A randomized parallel study design was used, with each of the two groups (n=14 & n=12) further randomised to receive either one of two test regimens in a crossover design in which each child acted as his/her own control. Iron bioavailability was measured with a stable-isotope technique based on erythrocyte incorporation 15 days after intake.

**Results:** The mean absorption of iron from ferrous sulfate and ferrous fumarate from the maize porridge meal was 6.85% (range 1.63-12.3%) and 9.29% (range 4.78-17.2%) respectively. The mean absorption of iron from ferrous sulfate and NaFeEDTA from the maize porridge meal was 6.81% (range 1.58-18.7%) and 11.51% (range 5.50-21.7%) respectively.

**Conclusion:** Assuming a realistic daily consumption of around 164g maize meal per day, at the current South African iron fortification level of 35ppm, ferrous fumarate at a 9% absorption level will contribute 0.52mg absorbed iron per day and NaFeEDTA at a 11% absorption level will contribute 0.63mg absorbed iron per day. Both ferrous fumarate and NaFeEDTA are sufficiently bioavailable from a maize porridge meal to provide a physiologically important amount of iron should they be used as fortificants in maize flour fortification.


**KEY WORDS:** Iron bioavailability, stable isotopes, ferrous fumarate, NaFeEDTA, sodium iron ethylenediaminetetraacetec acid, maize meal, food fortification

## INTRODUCTION

Iron deficiency is the most prevalent nutritional disorder in the developing world (1). Anaemia, 85% of which has been attributed to iron deficiency, occurs in more than half of the population of preschool children in developing countries compared with 10-11% in developed countries (2). According to a South African Vitamin A Consultative Group (SAVACG) report of 1995, 21% of South African children (6-71 months) are anaemic (3). The more recent first National Food Consumption Survey (NFCS) of 1999, shows that the mean dietary iron intake of children aged 1-9 years is less than 67% of the dietary reference intake for their age group (4).

South Africa's mandatory fortification of maize meal and wheat flour came into effect in October 2003. The NFCS found that maize and sugar are the foods most frequently consumed, with 75-95% of South African children regularly consuming these products. Additionally, up to 65% of these children regularly consume tea, whole milk, brown bread and margarine (4). Based on their widespread use across all income and age groups, as well as the relative ease of ensuring compliance to the regulations due to the small number of producers involved, maize meal and wheat flour were chosen to be fortified by the South African Food Fortification Programme. The national fortification programme is currently fortifying maize meal and wheat flour with elemental iron, specifically electrolytic iron (5).

Elemental iron powders are the most common iron fortificants used worldwide, because they cause the fewest organoleptic and stability problems in food products and are relatively inexpensive. However, research studies conducted over the past 45 years have produced highly variable results with respect to the bioavailability of these powders (from 5% to 145% relative to ferrous sulfate) (6). The South African Food Fortification Programme is considering other iron fortificants that might be more effective in alleviating iron deficiency, specifically ferrous fumarate and sodium iron ethylenediaminetetraacetic acid (NaFeEDTA).

The selection of these fortificants is, however, not without complications. Ferrous fumarate may cause unwanted colour and flavour reactions, but to a lesser extent than ferrous sulfate, and it has a similar, or slightly lower relative bioavailability value than ferrous sulfate in healthy adults (7). Ferrous fumarate does not cause undesirable sensorial changes in wheat flour at concentrations of up to 60mg/kg (8). NaFeEDTA appears highly suitable and an ideal iron fortificant for the fortification of cereal-based

foods, since it protects iron from phytate, does not promote fat oxidation in stored wheat flour and is stable during processing and storage (9). Iron from NaFeEDTA forms a common pool with nonheme iron (10,11), and this improves the absorption of nonheme iron sources (10,12), indicating that NaFeEDTA favourably modifies the characteristics of the nonheme iron pool (10). NaFeEDTA, however, has disadvantages such as causing undesirable changes in the sensorial properties of wheat and nixtamalized (lime-treated) maize flours, since NaFeEDTA or EDTA alone affects the dough viscosity of wheat flour and specific volume of bread (8). Consumer tests carried out by Kuyper on maize meal and wheat flour fortified with 18 mg Fe/kg NaFeEDTA have also revealed colour and taste differences in cooked porridge and bread. NaFeEDTA costs about 6-8 times more than ferrous sulfate in terms of equivalent amounts of absorbed iron, and this has limited its widespread use (13).

The aim of the present study was to measure the bioavailability of ferrous fumarate and NaFeEDTA from maize meal porridge in young children, which would assist in selecting a bioavailable alternative to elemental iron.

## SUBJECTS AND METHODS

### ❑ Subjects

Thirty black African children (17 boys and 13 girls), aged 4-6 years, were recruited from a nursery school in the nearby township (Potchefstroom, North West Province, South Africa) to participate in the study with their parents'/guardians' written consent. Children with haemoglobin values between 9–12 g/dl were included in the study. Each child was dewormed before the start of the study. The study was executed at the Metabolic Unit of the Nutrition Department (North-West University, Potchefstroom Campus). Ethical approval for the study was obtained from North-West University (Potchefstroom Campus) and the Baylor College of Medicine ethics committees.

Twenty-six subjects completed the study. Of the four dropout participants, three subjects did not show up at the start of the study, and one child's blood could not be collected.

### ❑ Meal preparation and administration

Maize meal (Mooirivier Mill, Potchefstroom, South Africa) was fortified with the mandatory vitamin and mineral premix without the elemental iron. The composition of the vitamin and mineral premix in the used maize flour per 1kg flour: 2085 µg RE

vitamin A, 2.1875mg thiamine, 1.6875mg riboflavin, 25mg niacin, 3.1250mg pyridoxine, 2mg folic acid, 35mg iron and 15mg zinc (5).

The maize meal was prepared as a soft porridge similar to that typically fed at the nursery school. Maize meal was added to boiling water, stirred and left until sufficiently cooked into a soft consistency such that individual 380g portions consisted of 86g maize meal and 480ml boiling water, the same amount normally consumed by children at the nursery school. Two teaspoons of sugar were added to each portion of porridge.

Solutions of stable isotopes of iron (3.0mg $^{57}$FeEDTA, 3.0mg $^{57}$Fum or 1.5mg $^{58}FeSO_4$) were added with a syringe to smaller amounts of hot porridge (20g) in small glass containers and refrigerated overnight. The porridge was reheated before being served.

The subject consumed the porridge for breakfast after an overnight fast under supervision. The research team fed the small amounts of porridge (20g) with the added isotope to the children before serving the bowl of porridge (360g). This was necessary to insure that each subject consumed the entire dose of stable isotope. Each container was rinsed with double distilled deionised water and given to children to drink to ensure that no isotope remained on the container.

- **Preparation of stable isotopes**

The stable isotopes were prepared at the Nutrition Department of the North-West University (Potchefstroom Campus). Stable iron isotopes ($^{57}$Fe and $^{58}$Fe) were purchased from Chemotrade GmbH & Co.KG, Düsseldorf, Germany, with isotopic enrichments of 95.4 and 92.8% respectively. NaFeEDTA stable isotopes were prepared in accordance with the published method of Loots and Colleagues (14). Briefly, 15 mM HCl (TraceSelect - Sigma) was added to 1mM stable iron isotope ($^{57}$Fe) in a 25ml conical flask which was pre-acid washed. The solution was placed on a heating block and heated slightly, with the stable iron isotope block serving as the magnetic stirrer, and a watch glass was placed over the opening. Hydrogen peroxide - - 7 mM (TraceSelectUltra – Sigma) was added, and the chlorine gas generation was allowed to take place to completion by means of slight heating. $Na_2EDTA.2H_2O$ (BiochemikaUltra – Sigma) pre-dissolved in 2ml of a 5mM NaOH (SigmaUltra – Sigma), prepared with double deionised sterile water, was added drop-wise in such a manner that a molar ratio of 1:1 Fe: EDTA was obtained. The resulting Na$^{57}$Fe

(III)EDTA solution was made up to the desired concentration (10mg Fe/ml) with double distilled deionised sterile water.

$^{57}Fe$ labelled ferrous fumarate was synthesized by Trace Sciences International as the powder. It was weighed to the nearest 0.1 mg, and added directly to the porridge without modification. Iron isotope solution was prepared in the same manner as the sulfate, using the methods described by Kastenmayer *et al.* (15). The metal was dissolved in 0.03 mL of 7M nitric acid and 0.125 mL of 0.5M sulphuric acid for every mg of elemental iron. The solutions were dried at 120°C, at 230°C, and finally at 500°C for 30 minutes in a sand bath or furnace. After cooling, the final products were re-suspended in 0.2M sulphuric acid at 0.240 mL for every mg of iron. Deionised water was added to produce a solution yielding a unit dose of iron in the form of $^{58}FeSO_4$ for each 2.5 mL of liquid.

- **Study design**

Subjects were randomized according to their haemoglobin values into 2 groups (**Figure 1**). Each group was further randomised to receive either one of two test regimens in a crossover design in which each child acted as his/her own control. Iron absorption from ferrous fumarate and NaFeEDTA in fortified maize meal porridge was studied. Ferrous sulfate was used as a reference compound.

The first study group completed regimen 1. Fe absorption from ferrous fumarate and ferrous sulfate fortified maize meal porridge was studied. Differently labelled test meals were fed on day 1 and 2. One test meal contained maize meal porridge fortified with ferrous fumarate labelled with $^{57}Fe$ and the other test meal contained maize meal porridge fortified with ferrous sulfate labelled with $^{58}Fe$. Test meals were fed after an overnight fast, under standardised conditions. Half of the children were given a breakfast of 380g cooked maize meal porridge and 1.5 mg elemental iron as ferrous sulfate on Day 1 and an identical breakfast, but with 3.0 mg of labelled ferrous fumarate as the iron source on Day 2. The other half were given a breakfast of 380g cooked maize meal porridge and 3.0 mg of labelled ferrous fumarate on Day 1, and an identical breakfast, but with 1.5 mg elemental iron as ferrous sulfate as the iron source on Day 2. Deionised water was served as a drink. No intake of foods or fluids was allowed for 3 hours after the test-meal intake.

In regimen 2, the same procedure was followed as in regimen 1, except that the second study group received 3.0 mg of labelled NaFeEDTA, instead of ferrous fumarate.

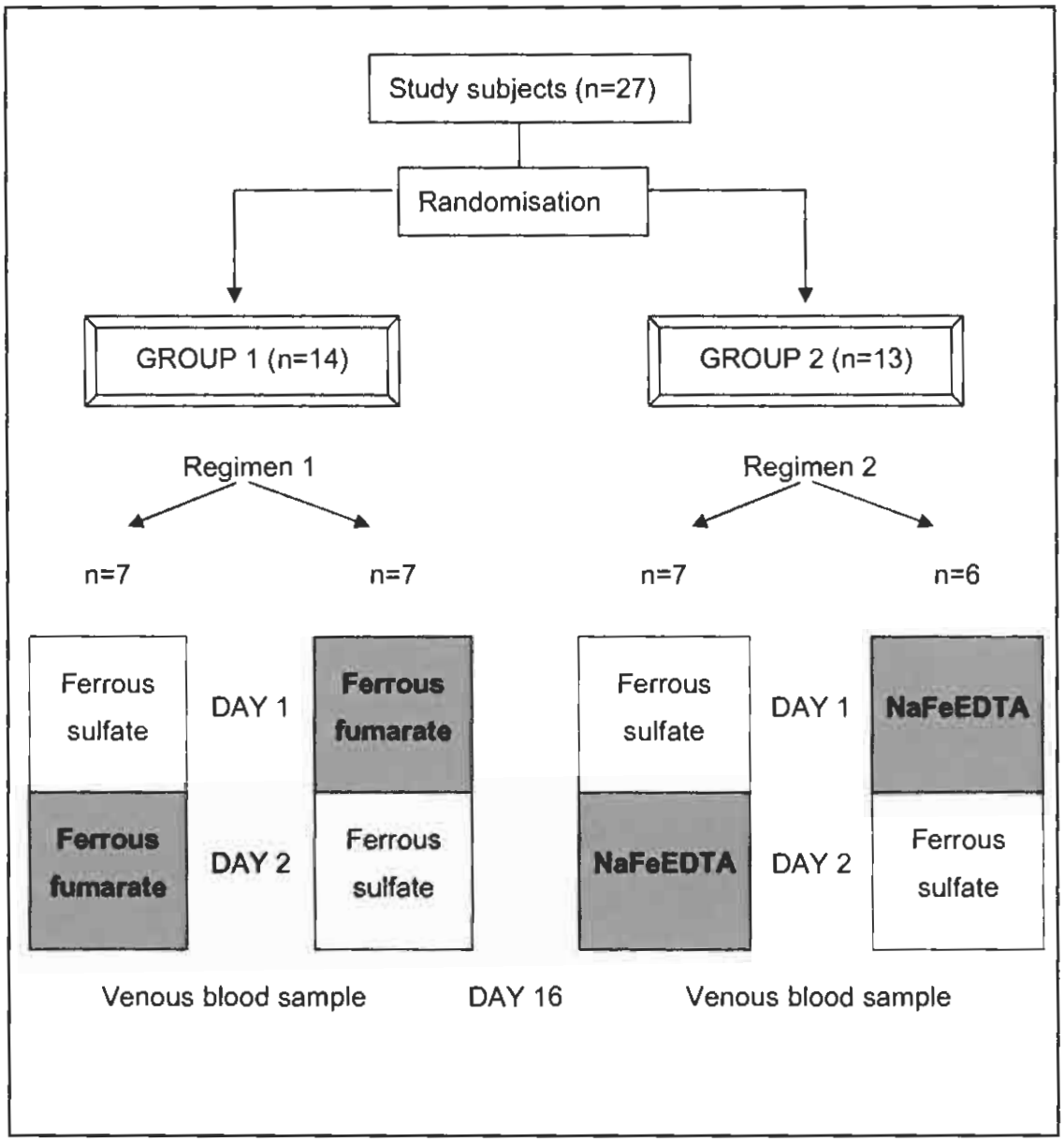


**Figure 1. Schematic presentation of study design**

- **Maize analysis *(phytate and iron)***

Phytate content was determined in accordance with the method of Wheeler and Ferrel (16) using 5g of maize. Fe content was determined as described by Doner and Ege (17) by means of flame atomic absorbance spectrometry conducted by the South African Grain Laboratory in Pretoria (South Africa).

- **Blood sampling and analysis**

A venous blood sample was drawn 14 days after intake of the second test meal in each regimen (Day 16) after an overnight fast. Blood was collected in 1ml EDTA vacutioners for the complete blood count measurements, and in 5ml plain vacutioners to determine isotope iron enrichment in red blood cells and iron status parameters. The iron status parameters analysed included serum iron, ferritin, transferrin and percentage transferrin saturation (Pathcare Pathologists, Klerksdorp, South Africa). Complete blood count measurements were analysed by the Beckman Coulter ACT 5Diff analysers. Haemoglobin values were adjusted for ethnicity (-10g/L) and altitude (+4g/L) (18).

Iron bioavailability was measured with a stable-isotope technique based on erythrocyte incorporation 15 days after intake. The samples were analysed at the Children's Nutrition Research Centre, Department of Pediatrics, Baylor College of Medicine, Houston, USA. Two microlitres of RBC were mixed with 30 microlitres of pure concentrated nitric acid in a clean Teflon container, and digested on a hot plate for about 5 minutes. The well digested solution was diluted to 3 millilitres with deionised water, and the solution was analysed for iron isotope ratios using a high resolution inductively coupled plasma mass spectrometer (19). A volume of 80 mL/kg was used as an estimate for the children's blood volume. The actual dietary iron absorbed by the body was reported as the endpoint. This was calculated by dividing the fraction of isotope that was incorporated into red blood cells ($RBC_{inc}$) by 0.9, assuming that 90% of all the iron absorbed from the diet is incorporated into red blood cells (20).

- **Statistical analysis**

The computer software package Statistica® was used for the statistical analysis of the data. Descriptive statistics (mean and SD) were calculated for all variables. The primary comparison was iron incorporation into red blood cells of ferrous fumarate and NaFeEDTA compared to ferrous sulfate. Results of iron absorption ratios from the two

iron sources studied were compared by means of a t-test for independent samples, thus comparing means in both test meals. Correlation analysis was used to relate serum ferritin, as a measure of iron status, to iron absorption from ferrous fumarate, NaFeEDTA and ferrous sulfate. Correlation analysis was also used to relate serum ferritin to absorption ratios of NaFeEDTA and ferrous fumarate against ferrous sulfate.

## RESULTS

### ❑ Subject characteristics

The subjects' iron status was low but still within the normal ranges for their age group. Two subjects (1 in each group) had iron deficiency anaemia (haemoglobin<11.5 g/dL; serum ferritin<15ug/L) and ten subjects (4 in Group 1; 6 in Group 2) were iron deficient (serum ferritin <15 ug/L). The mean serum ferritin concentration of the group was 18.3$\mu$g/L (SD 9.1 $\mu$g/L, range 4-37 $\mu$g/L). Individual haemoglobin concentrations ranged from 10.1 to 13.0 g/dL, with a mean of 11.8 g/d (SD 0.76 g/dL). The characteristics of the two groups that participated in the study are shown in **Table 1**.

**Table 1. Characteristics of subjects in each group**

| | **Group 1 ($FeSO_4$ & NaFeEDTA) n=14** | **Group 2 ($FeSO_4$ & FeFum) n=12** | **p-value between groups** |
|---|---|---|---|
| | **Mean (±SD)** | Mean (±SD) | |
| Age (mo) | **66.4 (4.40)** | 66.4 (5.58) | **0.986** |
| Height for age z-score | **-0.16 (1.16)** | -0.58 (0.93) | **0.318** |
| Weight for age z-score | **0.00 (1.68)** | -0.28 (1.02) | **0.622** |
| Weight for height z-score | **0.10 (1.57)** | 0.09 (0.93) | **0.985** |
| Serum iron ($\mu$mol/L) | **15. 6 (7.12)** | 14.2 (7.75) | **0.649** |
| Serum ferritin ($\mu$g/L) | **19.9 (10.3)** | 16.4 (7.54) | **0.348** |
| % Transferrin saturation | **26.8 (13.5)** | 23.4 (12.5) | **0.514** |
| Serum transferrin (g/L) | **2.68 (0.53)** | 2.70 (0.34) | **0.927** |
| Haemoglobin (g/dL) | **11.73 (0.86)** | 11.95 (0.63) | **0.468** |

$FeSO_4$: Ferrous sulfate, NaFeEDTA: Sodium iron EDTA; FeFum: Ferrous fumarate

- **Food analysis**

Each 100g raw maize meal had a mean (±SD) native Fe content of 1.33 mg (2.04) and a mean phytate content of 1171 mg.

- **Iron absorption from maize meal porridge**

The percentage of iron that was absorbed from labelled ferrous sulfate, ferrous fumarate and NaFeEDTA administrated with maize meal porridge is shown in **Table 2**. The mean absorption of iron from ferrous sulfate and ferrous fumarate from the maize porridge meal was 6.85% (range 1.63-12.3%) and 9.29% (range 4.78-17.2%) respectively, and 6.81% (range 1.58-18.7%) and 11.51% (range 5.50-21.7%) from ferrous sulfate and NaFeEDTA respectively. The mean iron absorption from ferrous fumarate and NaFeEDTA was 9.29% and 11.51% respectively, with no significant difference in iron absorption observed between the two compounds (p=0.19).

**Table 2** presents absorption ratios of the 2 fortificants, NaFeEDTA and ferrous fumarate, against the reference dose ($FeSO_4$). The mean NaFeEDTA/$FeSO_4$ absorption ratio of 2.83 (range 0.66-6.96) does not differ significantly (p=0.214) from the mean FeFum/FeSO4 absorption ratio of 1.88 (range 0.66-5.44) (**Figure 2**).

**Figure 3** illustrates the relation between serum ferritin and the percentage of iron absorption from ferrous sulfate, ferrous fumarate and NaFeEDTA. A significant inverse relation was found between iron absorption from NaFeEDTA and serum ferritin concentration, but not for iron absorption from ferrous fumarate or ferrous sulfate.

**Figure 4** demonstrates the relation between serum ferritin and the absorption ratios of NaFeEDTA and ferrous fumarate against ferrous sulfate.

**Table 2.** **Percentage iron absorption from ferrous sulfate ($FeSO_4$), ferrous fumarate (FeFum) and Sodium iron EDTA (NaFeEDTA) in maize meal porridge**

| Iron absorption | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group 1 | | | | Group 2 | | | |
| Subject NR | $FeSO_4$ | NaFeEDTA | NaFeEDTA / $FeSO_4$ | Subject NR | $FeSO_4$ | FeFum | FeFum / $FeSO_4$ |
| 1 | 18.7% | 14.6% | 0.78 | 16 | 12.28% | 8.13% | 0.66 |
| 2 | 9.31% | 6.42% | 0.69 | 17 | 8.48% | 13.00% | 1.53 |
| 3 | 10.2% | 8.96% | 0.88 | 18 | 3.74% | 6.24% | 1.67 |
| 4 | 3.83% | 8.24% | 2.15 | 19 | 7.23% | 6.04% | 0.84 |
| 5 | 6.82% | 10.5% | 1.53 | 20 | 11.54% | 10.70% | 0.93 |
| 6 | 3.98% | 5.50% | 1.38 | 21 | 10.39% | 9.99% | 0.96 |
| 7 | 8.10% | 9.45% | 1.17 | 23 | 3.16% | 4.78% | 1.51 |
| 8 | 17.0% | 11.2% | 0.66 | 25 | 4.91% | 5.79% | 1.18 |
| 9 | 3.12% | 21.7% | 6.96 | 26 | 4.56% | 12.03% | 2.64 |
| 10 | 3.03% | 10.3% | 3.41 | 28 | 1.63% | 8.85% | 5.44 |
| 11 | 4.39% | 19.7% | 4.47 | 29 | 12.05% | 17.21% | 1.43 |
| 12 | 1.96% | 12.8% | 6.54 | 30 | 2.30% | 8.72% | 3.80 |
| 13 | 1.58% | 7.55% | 4.77 | | | | |
| 14 | 3.35% | 14.3% | 4.26 | | | | |
| **Mean** | **6.81%** | **11.5%** | **2.83** | | **6.85%** | **9.29%** | **1.88** |
| SD | 5.40% | 4.73% | 2.22 | | 3.98% | 3.58% | 1.42 |

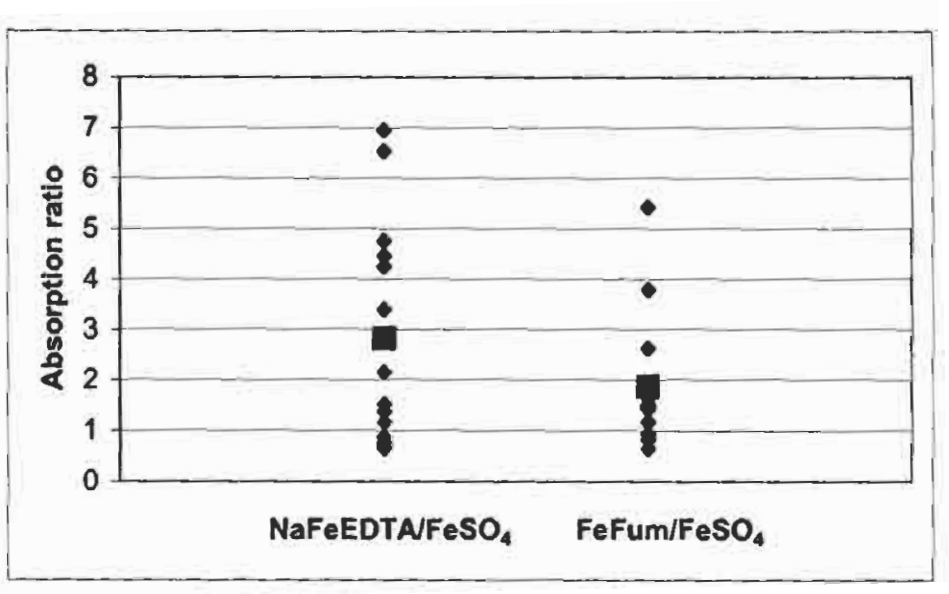


**Figure 2** **Absorption ratios of Sodium iron EDTA (NaFeEDTA) and ferrous fumarate (FeFum) against ferrous sulfate ($FeSO_4$) (■: mean)**

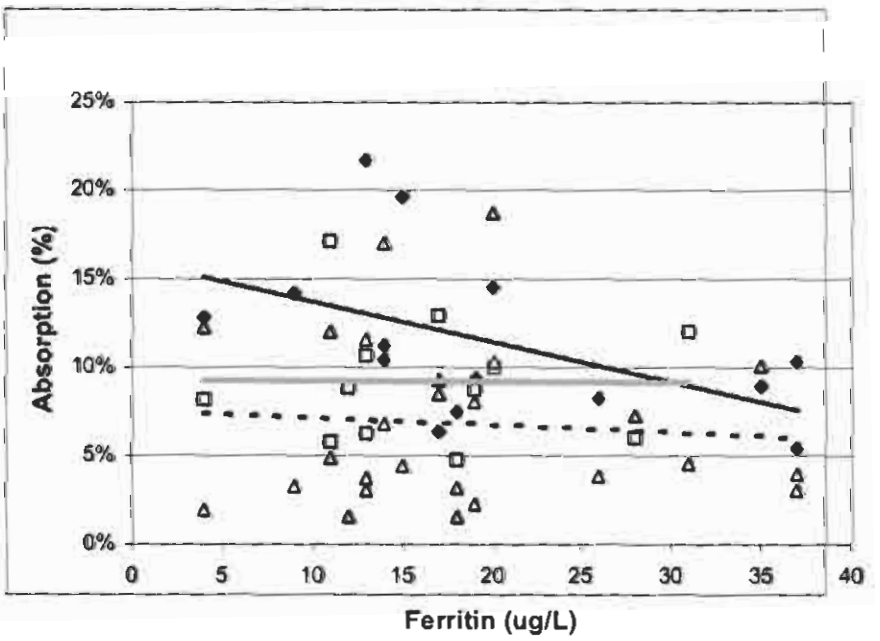


**Figure 3.** **Relation between serum ferritin and percentage iron absorption from ferrous sulfate (– –△; r=0.01, p=0.944), ferrous fumarate (□; r=0.03, p=0.922) and Sodium iron EDTA (—◆; r=0.59, p=0.025).**

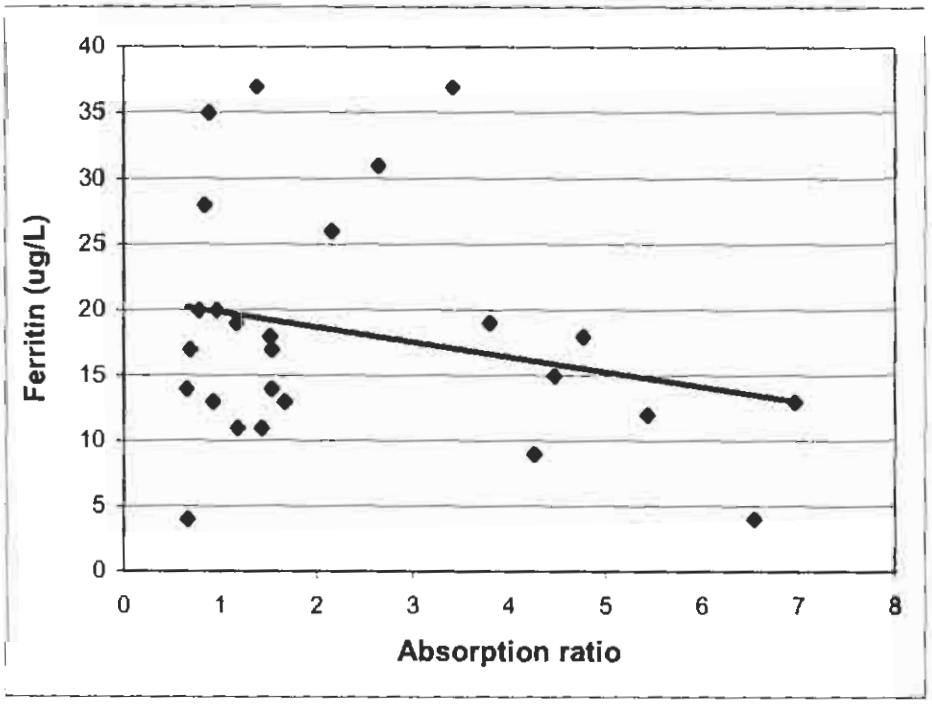


**Figure 4.** **Relation between serum ferritin and the absorption ratios of NaFeEDTA and ferrous fumarate against ferrous sulfate (r=-0.17, p=0.404).** [Relation between serum ferritin and absorption ratio of NaFeEDTA against ferrous sulfate (r=-0.34, p=0.240). Relation between serum ferritin and absorption ratio of ferrous fumarate against ferrous sulfate (r=0.18, p=0.566)].

## DISCUSSION

Maize is the primary staple for an estimated 360 million people living in sub-Saharan Africa. This is the first study to examine the bioavailability of ferrous fumarate and NaFeEDTA from South African maize meal in black African children using stable isotope technology.

The mean iron absorption from the reference dose of ferrous sulfate was almost identical in the two groups (6.81% and 6.85%), and there was no significant difference between iron bioavailability from ferrous fumarate and NaFeEDTA. These results suggest that ferrous fumarate and NaFeEDTA could both be effective sources of iron in diets that are rich in phytates (1171mg phytate/100g).

The fact that iron absorption from ferrous fumarate (9.3%) is close to that of NaFeEDTA (11.5%) is somewhat surprising, since two previous studies showed a 2-3 and 6 fold higher iron absorption from NaFeEDTA compared to ferrous fumarate in infant cereal (122-770 mg phytate/100g) (9) and corn-masa tortillas (~550 mg phytate/100g) (21) respectively. The differences in food matrix between studies, as well as the phytate content of the test meals could possibly explain this finding. According to Dary (8), absorption patterns depend not only on the nature of the iron compound, but also to a great extent on the food matrix, and conclusions should not be extrapolated from one substrate to another.

In a study by Hurrell *et al.* (9), it was suggested that iron absorption is also influenced by the amount of phytate present in the food matrix. The study found that iron absorption from NaFeEDTA in a high-extraction-wheat bread roll (3.91%) was much lower than a low-extraction-wheat roll (11.5%) in which phytate was degraded to zero (9). The phytate content of the maize meal used in the present study was much higher (1171mg phytate/100g) than the previous studies (122-770 mg phytate/100g) that showed 2-6 fold higher iron absorption from NaFeEDTA compared to ferrous fumarate. The high phytate content of the maize meal used in the present study could, therefore, have caused lower iron absorption from NaFeEDTA.

One limitation of this study is the fact that the dose of ferrous sulfate (1.5 mg) was different from ferrous fumarate and NaFeEDTA (3mg). The intention was to use 3 mg in each serving, but a procedural error occurred (no extra iron was added) with the first batch of ferrous sulfate samples. Although this is certainly a limitation of the study, the test was for the bioavailability of fumarate vs. NaFeEDTA using ferrous sulfate as a reference only and, therefore, it does not change the outcome of the primary objective. Both ferrous fumarate and NaFeEDTA were compared to the same (albeit smaller) dose. No direct comparisons can be made between ferrous sulfate and ferrous fumarate or NaFeEDTA in this study due to the difference in iron dose.

Since the iron content of the test meals was different, the data are normalized to iron absorption from the reference meal and are not "relative bioavailability" values, but normalised iron absorption values. This data should, therefore, not be compared to other studies that reported relative bioavailability values.

NaFeEDTA seems to be the most bioavailable option, but NaFeEDTA has been found to affect the sensory properties of wheat flour and maize meal (8). An unpublished

study by Alvarado found that the upper-level sensory threshold (ppm) for NaFeEDTA in white wheat flour is 15 mg/kg (8). However, according to Rodenstein, maize meal fortified with 20 mg Fe/kg NaFeEDTA is being marketed successfully in Kenya (13). Efficacy trials have used fortificant levels of 28 and 56 mg/kg NaFeEDTA in whole maize (22) and 20 mg/kg NaFeEDTA in wheat flour (23). Both trials showed significantly higher efficacy in improving iron status in children compared to electrolytic iron (22,23). The cost of NaFeEDTA might be a concern for use as iron fortificant in fortification programmes, but less NaFeEDTA would be used to achieve the same amount of absorbed iron as when ferrous fumarate is used. The price of NaFeEDTA would also probably fall when the use and demand thereof increases.

Although ferrous fumarate does not cause undesirable sensorial changes in wheat flour at concentrations of up to 60mg/kg, ferrous fumarate-fortified chocolate drink powder reconstituted with boiling water or milk (>80°) has, however, changed colour from red/brown to an unacceptable gray (24). Unacceptable colour changes could, therefore, occur if maize meal fortified with ferrous fumarate is mixed with boiling milk or water.

Since maize meal for porridge and wheat flour for bread are both fortified with the same iron fortificant in South Africa, it is important to determine the maximum possible amount of NaFeEDTA that will not cause any undesirable changes to both products. The small advantage of NaFeEDTA over ferrous fumarate from a bioavailability point of view, as found in this study, can be reduced due to the maximum possible amounts of NaFeEDTA that could be loaded into both wheat flour and maize meal (8).

An inverse relation was found between iron absorption from NaFeEDTA and iron status, expressed as serum ferritin concentration, but not for iron absorption from ferrous fumarate or ferrous sulfate. Mendoza et al. (25) observed a similar trend in their assessment of iron absorption from unmodified maize and genetically altered, low-phytate maize. Their study also found a significant inverse relation between serum ferritin and iron absorption for the individual diets fortified with NaFeEDTA, but no significant correlations of the diets fortified with ferrous sulfate. According to Mendoza *et al.* (25), the low absorption of iron (1.69 and 1.91) and limited variability of absorption with these diets are possible reasons for this observation. The absorption of iron from ferrous sulfate is not as low in the present study (6.81% and 6.85%), however, and this suggests that factors other than low absorption and variability contribute to the lack of a

correlation between iron absorption from ferrous sulfate (and probably ferrous fumarate) and serum ferritin.

No indicator of infection was measured in this study and, therefore, no children with infections were identified and excluded from the study. The presence of infections in some of the children could not be a possible explanation for the lack of inverse relation found between iron absorption from ferrous sulfate and ferrous fumarate, and iron status, because iron absorption from NaFeEDTA and ferrous sulfate was measured in the same group of children.

The contribution of NaFeEDTA and ferrous fumarate to the iron requirements of young children was calculated using the iron bioavailibility of the compounds determined in this study. Assuming a realistic daily consumption of around 164g dry maize meal per day at the current South African iron fortification level of 35ppm, ferrous fumarate at a 9% absorption level will contribute 0.52mg iron per day while NaFeEDTA at an 11% absorption level will contribute 0.63mg iron per day. The requirement for absorbed iron among children aged 4-8 years is 0.74mg/day (26). Ferrous fumarate and NaFeEDTA will, therefore, respectively provide 70% and 85% of the daily absorbed iron requirements for children aged 4-8 years when consuming 164g dry maize meal per day.

In conclusion, these results indicate that both ferrous fumarate and NaFeEDTA are sufficiently bioavailable from a maize porridge meal to provide a physiologically important amount of iron should they be used as fortificants in maize meal fortification. Organoleptic trials as well as a cost-benefit analysis is needed in order to make a final decision, but was beyond the scope of this investigation.

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# CHAPTER 4

# GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 4.1 INTRODUCTION

The aim of this study was to determine the bioavailability of ferrous fumarate and NaFeEDTA from maize meal porridge in younger children, which would assist in selecting a bioavailable alternative to electrolytic iron in the South African National Food Fortification Programme. This final chapter provides a summary of the main findings as found in the experimental study, supported by findings from the literature. As the results of the study are discussed, interpreted, elucidated and compared to the relevant literature in the preceding chapter, only a general conclusion will subsequently be made. This will be followed by general recommendations regarding this study as deduced from these findings.

## 4.2 MAIN FINDINGS

### 4.2.1 Ferrous fumarate as alternative iron fortificant

The mean absorption of iron from ferrous fumarate was 9.3% from maize meal porridge in young children. In terms of bioavailability, ferrous fumarate did not differ significantly from NaFeEDTA. Ferrous fumarate fortified at 35ppm could contribute 70% of the absorbed iron requirements of 4-8 year old children per day, assuming a daily consumption of 164g dry maize meal. Ferrous fumarate is organoleptically more stable than NaFeEDTA in maize meal and wheat flour. Ferrous fumarate does not cause undesirable sensorial changes in wheat flour and nixtamalised corn flour when added up to 60mg/kg and 30mg Fe/kg respectively (Dary, 2002). Ferrous fumarate could, therefore, be an effective alternative source of iron in diets that are rich in phytates.

### 4.2.2. NaFeEDTA as alternative iron fortificant

The mean absorption of iron from NaFeEDTA was 11.5% from maize meal porridge in young children. NaFeEDTA fortified at 35ppm could contribute 85% of the absorbed iron requirements of 4-8 year old children per day, when consuming 164g dry maize meal per day. NaFeEDTA still has a slight advantage over ferrous fumarate, having a 20% higher iron absorption. This advantage could be compromised by technological difficulties that exist around NaFeEDTA, such as undesirable sensorial changes in cooked maize meal (porridge) and wheat flour (bread) (Kuyper as quoted by Bothwell & MacPhail, 2004). Efficacy trials showed improvements in iron status when NaFeEDTA is used as the iron fortificant in whole maize flour and wheat flour, using fortificant levels of 28 mg/kg, 30 mg/kg and 56mg/kg (Adang'o *et al.*, 2006; Chen, 2006).

## 4.3 CONCLUSION

Maize meal is the staple food of most South Africans. The high prevalence of iron deficiency and anaemia among children highlights the need for iron fortification, especially with a highly bioavailable iron compound. Water insoluble compounds such as elemental iron are the least absorbable forms of iron, and although they do not cause any organoleptic problems in maize and wheat flour, their bioavailability and efficacy to improve iron status is questionable. Results from the iron bioavailability study show that NaFeEDTA and ferrous fumarate are both sufficiently bioavailable from a maize based meal rich in phytate. Both NaFeEDTA and ferrous fumarate could contribute significantly to the iron requirements of young children, even at the low current South African iron fortification level of 35ppm.

Given the data available, it is clear that electrolytic iron could be replaced either with ferrous fumarate or with NaFeEDTA. The choice between ferrous fumarate and NaFeEDTA as when it comes to finding the alternative iron fortificant will depend on factors such as technical compatibility, measured bioavailability, relative cost and organoleptic characteristics.

## 4.4 RECOMMENDATIONS

- This is the first stable isotope study measuring iron bioavailability from South African maize meal. Because the National Food Fortification Programme is also fortifying wheat flour with the same vitamins and minerals, it is recommended that a similar study should be conducted to determine the bioavailibility of the iron compounds, ferrous fumarate and NaFeEDTA from wheat flour.

- It is recommended that technological compatibility studies should be carried out to determine the maximum amount of ferrous fumarate and NaFeEDTA that can be added to maize meal and wheat flour without organoleptic influences.

- When technical compatibility studies are done, comparative cost analysis should be conducted with the maximum load of iron compound in the food vehicle, thus to determine the most cost effective compound by taking into consideration the price of iron compound, percentage absorption in the food vehicle and thereby calculate the total fortification cost and the percentage of absorbed iron provided by a given portion of the food vehicle.

- Efficacy studies should be conducted to demonstrate that regular consumption of fortified maize has a beneficial effect on the iron status of the target population.

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