DUAL FORTIFICATION OF SALT WITH IODINE AND IRON IN AFRICA

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Salt dual fortified with iodine and micronized ferric pyrophosphate improves iron status but not hemoglobin in children in Côte d'Ivoire

CONCLUSIONS AND PERSPECTIVES
ABBREVIATIONS

ACT \( \alpha_1 \)-antichymotrypsin
AGP \( \alpha_1 \)-acid glycoprotein
APP Acute phase protein
CRP C-reactive protein
DE Dextrose equivalent
DFS Dual fortified salt
EDTA Ethylenediaminetetraacetic acid
EGRAC Erythrocyte glutathione reductase activity coefficient
FAD Flavin adenine dinucleotide
FAO Food and Agriculture Organization
FePP Ferric pyrophosphate
FMN Flavin mononucleotide
Hb Hemoglobin
ICCID International Council for Control of Iodine Deficiency Disorders
ID Iron deficiency
IDA Iron deficiency anemia
IDD Iodine deficiency disorders
ILSI International Life Sciences Institute
INACG International Nutritional Anemia Consultative Group
IS Iodized salt
IVACG International Vitamin A Consultative Group
IZiNCG International Zinc Nutrition Consultative Group
MCV Mean corpuscular volume
MI Micronutrient Initiative
MSG Monosodium glutamate
NaFeEDTA Sodium iron Ethylenediaminetetraacetic acid
OPA Orthophosphoric acid
PUFA Polyunsaturated fatty acid
RBP Retinol binding protein
RBV Relative bioavailability
RDA Recommended Dietary Allowance
SF Serum ferritin
SFE-171 Ferrous sulfate encapsulated with soy lecithin
SHMP Sodium hexametaphosphate
SR Serum retinol
T\(_3\) Triiodothyronine
T\(_4\) Thyroxine
TfR Serum transferrin receptor
Tg Thyroglobulin
TIBC Total iron binding capacity
TSH Thyrotropin, Thyroid-stimulating hormone
UI Urinary iodine
UNICEF United Nations Children's Fund
UNU United Nations University
USI Universal salt iodization
VAD Vitamin A deficiency
WHO World Health Organization
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SUMMARY

Iron and iodine deficiencies affect more than one third of the world’s population, and are highly prevalent in developing countries. In West Africa, 1 in 5 school children suffers from both goiter and iron deficiency anemia (IDA) and IDA has been shown to reduce the efficacy of iodine prophylaxis in areas of endemic goiter such as in western Côte d’Ivoire. In countries, where existing food supplies and/or limited access fail to provide adequate levels of these nutrients in the diet, food fortification is a promising approach. Today, iodization of salt is highly effective in reducing or eliminating iodine deficiency disorders (IDD) in many African countries. However, there is no comparable, proven method for controlling IDA on a national level. Salt is one of very few food items consumed daily by rural African populations even in poor remote areas of subsistence farming. Therefore, dual fortification of salt with iodine and iron (Fe) using the existing infrastructure for iodization to also fortify salt with Fe could be an effective fortification strategy to combat IDD and IDA in regions with a high prevalence of these micronutrient deficiencies.

The aim of this thesis was to develop a stable (color and iodine content) and acceptable dual fortified salt, which does not cause off-color or off-flavor in typical Ivorian meals and which is efficacious in reducing the IDA prevalence in school children.

Local salt was fortified with iodine and different encapsulated and non encapsulated commercially available Fe compounds in temperate Morocco and tropical Côte d’Ivoire. Salts were stored for 6 months under local conditions and color change and iodine content were analyzed periodically. Acceptability was tested at the local market. Most of the dual fortified salts developed yellow or brown off-colors during storage and were judged as unacceptable in both countries. The best performing salt in both countries was salt fortified with ferric pyrophosphate (FePP) which resulted in an off-white color and acceptable iodine loss after storage. In general, encapsulation did not greatly reduce color change or iodine loss. These results suggest that encapsulation techniques need to be improved if encapsulated well bioavailable Fe compounds are used for salt fortification or to use FePP taking into account its low bioavailability.

The next step consisted in developing an encapsulation process that produces microcapsules containing iodine, micronized FePP, and retinyl palmitate and that
Summary

prevents adverse sensory reactions and iodine loss in salt. Micronutrients were encapsulated with hydrogenated palm fat by spray cooling and the produced microcapsules were added to local Moroccan salt and color change, iodine and vitamin A loss during production and storage for 6 months were determined. Color change and iodine loss of the fortified salt were acceptable and retinyl palmitate stability was excellent. Sensory tests with Moroccan dishes cooked with the fortified salt showed no difference compared to the same dishes prepared with non fortified salt. Although these results look promising and multiple micronutrient fortification is desirable in developing countries, encapsulation is a costly process and often fails when good bioavailable compounds are used. In addition encapsulation may decrease bioavailability.

To test the bioavailability of encapsulated FePP and the influence of particle size reduction of nonencapsulated FePP on bioavailability, a rat study was performed using the hemoglobin repletion assay. Weanling rats were fed a Fe deficient diet for 24 days. Anemic rats were fed the iron deficient diet fortified with one of the test Fe compounds (FePP with a mean particle size of \( \approx 21 \mu m, \approx 2.5 \mu m, \approx 2.5 \mu m \) encapsulated in palm fat, or \( \approx 0.5 \mu m \)) or ferrous sulfate for 14 days. Relative bioavailability (RBV) of the 0.5 \( \mu m \) compound was significantly higher compared to the other compounds and was not different from ferrous sulfate. The RBV of the 2.5 \( \mu m \) compound was higher (69%) than the 21 \( \mu m \) compound (59%), although this difference was not statistically significant. Encapsulation at a 60:40 capsule:core ratio resulted in a significant decrease of the RBV from 69 to 43%. These results suggest that by decreasing the particle size bioavailability of FePP may increase and make this compound more useful for food fortification.

In an efficacy study in a rural village in southern Côte d'Ivoire salt fortified with iodine and micronized FePP (mean particle size \( \approx 2.5 \mu m \)) (DFS) or iodized salt (IS) was distributed to the school children of the 4 schools and the head of the households were asked to use this salt for all food preparation. After 6 months of salt consumption Hb did not change in both groups but iron status indicators as well as body iron concentration significantly improved in the DFS group compared to baseline whereas no change was observed in the IS group. Both, the prevalence of iron deficiency anemia (IDA) and of iron deficiency (ID) without anemia was significantly reduced in the DFS group, whereas in the IS groups only IDA prevalence
was reduced. However, the prevalence of anemia did not change in both groups indicating that other causes besides Fe deficiency contributed to the high prevalence of anemia.

In conclusion, these studies suggest that salt dual fortified with iodine and micronized FePP is a promising approach to reducing iron and iodine deficiency in regions where anemia and goiter are common and fortification strategies are limited, but simultaneous combat of other micronutrient deficiencies and infections are crucial.
ZUSAMMENFASSUNG


Zusammenfassung

konnten durch die Verkapselung nur geringfügig vermindert werden. Diese Resultate zeigen, dass entweder die Verkapselungstechniken verbessert werden müssen, damit verkapselte, gut bioverfügbare Eisenverbindungen für die Salzanreicherung angewendet werden können oder dass Eisenpyrophosphat mit einer geringeren Bioverfügbarkeit eingesetzt werden muss.


Um den Einfluss von verkapseltem FePP und der Partikelgrösse von nicht verkapseltem FePP auf die Bioverfügbarkeit zu prüfen, wurde der Hämoglobin-Repletions-Test in Ratten durchgeführt. Frisch abgestillte Ratten wurden während 24 Tagen mit einem eisenarmen Futter ernährt. Die anämischen Ratten wurden dann während 14 Tagen mit demselben Futter, welches aber mit einer der zu testenden Eisenverbindungen (FePP mit einer mittleren Partikelgrösse von \( \approx 21 \mu m, \approx 2.5 \mu m, \approx 2.5 \mu m \) verkapselt in Palmfett und \( \approx 0.5 \mu m \)) oder mit Eisensulfat angereichert war, gefüttert. Die relative Bioverfügbarkeit (RBV) der 0.5\( \mu m \)-Verbindung war signifikant höher als die der anderen Testverbindungen und vergleichbar mit der von Eisensulfat. Die RBV der 2.5\( \mu m \)-Verbindung war höher (69%) als die der 21\( \mu m \)-Verbindung (59%), der Unterschied war aber nicht signifikant. Die Bioverfügbarkeit der verkapselten Verbindung (Verhältnis Kapsel:Substrat = 60:40) nahm im Vergleich zur nicht verkapselten Verbindung signifikant von 69 auf 43% ab. Diese Resultate
zusammenfassung

deuten darauf hin, dass durch die Verringerung der Partikelgröße die Bioverfügbarkeit von FePP eventuell erhöht werden kann, was diese Verbindung für die Anreicherung von Lebensmitteln attraktiver machen würde.


Zusammenfassend zeigen diese Studien, dass zweifach angereichertes Salz mit Jod und mikronisiertem Eisen in Regionen mit häufigem Vorkommen von Anämie und Kropf und wo die Auswahl an Lebensmitteln für die Anreicherung beschränkt ist, eine viel versprechende Strategie zur Verringerung von Eisen- und Jodmangel ist. Jedoch spielt die gleichzeitige Bekämpfung von anderen Mikronährstoff-Mangelercheinungen und Infektionen eine entscheidende Rolle.
INTRODUCTION

Iron deficiency anemia (IDA) and the iodine deficiency disorders (IDD) remain major public health problems in Africa and worldwide, and many children and pregnant women are at high risk for both goiter and IDA (WHO/UNICEF/ICCIDD, 2001; WHO/UNICEF/UNU, 2001). These deficiencies can lead to impaired growth and cognitive development, birth defects, cretinism, as well as decreased school and work performance and poor general health. Coexisting IDD and IDA are common in western Côte d'Ivoire, where 1 in 5 school children suffers from both goiter and IDA (Zimmermann et al., 2000b). In addition, it has been shown that the response to oral iodized oil and iodized salt is impaired in goitrous children with IDA, and provision of oral Fe supplements to goitrous children with IDA improves their response to iodine (Zimmermann et al., 2000b; Zimmermann et al., 2000a; Hess et al., 2002).

The main etiologic factors for iron (Fe) deficiency are low dietary Fe intake and low Fe bioavailability and therefore Fe fortification of the food supply in Côte d'Ivoire could be a sustainable approach to treating and preventing IDA (Staubli-Asobayire, 2000). Since 1998, iodized salt has become widely available in Côte d'Ivoire. Salt is also likely to be the only effective vehicle for Fe fortification, as it is the only commercial product widely and regularly consumed, even in poor, remote rural populations (Hess et al., 1999; Staubli-Asobayire, 2000). Therefore, dual fortification of the salt with iodine and Fe could be a cost effective and sustainable approach to preventing these disorders in Côte d'Ivoire. However, ensuring the stability and bioavailability of Fe and iodine when used in combination in salt is problematic (Sattarzadeh & Zlotkin, 1999). Interactions between iodine and Fe can accelerate iodine losses due to evaporation and catalytic oxidation of I to I$_2$ (Venkatesh Mannar, 1987; Diosady & Venkatesh Mannar, 2000). Fe may be oxidized to the ferric form, which usually has lower bioavailability and can give off-colors. Several dual fortified salts have been developed, but their acceptability may be limited due to undesirable darkening of the salt or losses of iodine.
Introduction

Objectives
The general aim of this thesis was to develop and test dual fortified salt as a vehicle for Fe and iodine in Côte d'Ivoire. The first objective was to test the stability (color and iodine content) of different forms of dual fortified salt under Ivorian conditions and if necessary to investigate further developmental work in order to produce a stable dual fortified salt and to test the effects of adding dual fortified salt on color and flavor of traditional Ivorian meals. The second objective was to test the bioavailability of iron compounds used to prepare dual fortified salt to determine the optimal level of iron fortification for dual fortified salt in Côte d'Ivoire. The final objective was to conduct an efficacy study to determine if salt dual fortified with iron and iodine can reduce the prevalence of IDA, and, by improving iron status, increase the efficacy of the iodine in the salt.

Outline of the thesis

Chapter 1: Literature review on micronutrient deficiencies in developing countries, on strategies to combat those and on possibilities to produce stable dual fortified salt.

Chapter 2: Stability and acceptability testing of different dual fortified salts to investigate whether encapsulation of Fe would help to overcome adverse sensory reactions in dual fortified salt in North and West Africa

Chapter 3: Development of stable microcapsules containing iron, iodine and vitamin A and testing of their stability and acceptability for use in salt fortification

Chapter 4: Rat study to evaluate the effect of particle size reduction and encapsulation on the bioavailability of ferric pyrophosphate

Chapter 5: Efficacy study to test whether salt dual fortified with iodine and micronized ferric pyrophosphate is efficacious in Ivorian school children
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CHAPTER 1 - LITERATURE REVIEW

MICRONUTRIENT DEFICIENCIES IN DEVELOPING COUNTRIES

Micronutrient deficiencies

Micronutrient deficiencies are a significant cause of malnutrition and associated ill health throughout the world (Mason et al., 2001). This is particularly true in the developing world, where nearly 20% of the population suffers from iodine deficiency disorders (IDD), about 25% of children have subclinical vitamin A deficiency (VAD) and more than 40% of women are anemic. These deficiencies often coexist in developing countries and in regions of West and North Africa 20-38% of school children may suffer from iron and iodine deficiency (Zimmermann et al., 2000b; Zimmermann et al., 2003b). Micronutrient deficiencies can lead to impaired growth and cognitive development, birth defects, cretinism, and blindness, as well as decreased school and work performance and poor general health (Mason et al., 2001).

Iron

More than any other metal, iron (Fe) is a key element in the metabolism of all living organisms (Fairbanks, 1998). The body requires Fe for the synthesis of the oxygen transport proteins hemoglobin and myoglobin, and for the formation of heme enzymes and other iron containing enzymes, which participate in electron transfer and oxidation-reduction reactions (Hurrell, 1997b). Almost two-thirds of iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store and the remaining 15% is found in the myoglobin of muscle tissue and in a variety of enzymes (Institute of Medicine, 2001). Since Fe loss is very limited (≈ 1 mg/d) normal Fe balance is maintained by regulation of Fe absorption (Fairbanks, 1998). Fe is actively absorbed in the duodenum, moved across the duodenal mucosa into the blood where it is transported by transferrin to the cells or the bone marrow for erythropoiesis and absorption is increased during deficiency and decreased when erythropoiesis is depressed (Hurrell, 1997b). The adult male requires ≈ 1 mg Fe/d, menstruating women up to 1.4 mg/d (Hurrell, 1997b). Excess
Chapter 1

Iron is stored as ferritin or hemosiderin in the liver, spleen and bone marrow (Hurrell, 1997b).

There are two kinds of dietary Fe: heme Fe and non-heme Fe (Hallberg, 1981). The primary sources of heme Fe are the hemoglobin and myoglobin from consumption of meat, poultry, and fish whereas non-heme Fe is obtained from cereals, pulses, legumes, fruits, and vegetables (FAO/WHO, 2001b). Heme Fe is highly bioavailable (15-35%) (Monsen et al., 1978) and little affected by dietary factors whereas non-heme Fe absorption is strongly influenced by the presence of other food components and can range from less than 1% to almost 100% (Hurrell, 1997b). Inhibitors such as phytic acid, polyphenols, calcium and peptides from partially digested proteins bind Fe in complexes from which it is unavailable for absorption. Enhancers such as ascorbic acid and muscle tissue may reduce ferric Fe to ferrous Fe and bind it in soluble complexes which are available for absorption. The equilibrium of this inhibitory and enhancing factors and the Fe status of the subject determines the bioavailability of Fe from a meal (Hurrell, 1997b).

Although the body has mechanisms for maintaining Fe balance and preventing iron deficiency (ID) and overload, such as continuous re-utilization of Fe from catabolized erythrocytes, access to the specific storage protein, ferritin, which can store and release Fe to meet excessive Fe demands and the regulation of absorption from the intestine which is increased during ID, it can only balance losses up to a certain point beyond which ID will develop (FAO/WHO, 2001b). In developing countries, where intake of heme iron and ascorbic acid are often low and staple foods often contain a high amount of phytic acid, people are at high risk to develop ID. The first stage of ID is depleted Fe stores, followed by iron-deficient erythropoiesis and finally the most severe form of iron deficiency anemia (IDA) (Nestel & Davidsson, 2002). During the first two stages, called ID, hemoglobin (Hb) concentration remains above the established cut-off levels whereas during the third stage (IDA) it falls below. In 2003 WHO stated that ID is the most common nutritional disorder in the world with an estimated 66-80% of the world's population may be Fe deficient and over 30% being anemic, mainly due to Fe deficiency (WHO, 2003a). The highest prevalence is found in infants, children, adolescents, and women of childbearing age, especially pregnant women (FAO/WHO, 2001b). Iron deficiency adversely affects the cognitive performance, behavior, and physical growth of infants, preschool and school-age children, the immune status and morbidity from infections of all age groups and the
physical capacity and work performance of adolescents and adults (WHO/UNICEF/UNU, 2001). Specially, IDA during pregnancy increases perinatal risks for mothers and neonates and overall infant mortality. Although anemia is usually caused by ID, several micronutrient deficiencies in addition to iron can cause anemia as well as infections and parasites (Allen & Casterline-Sabel, 2001). Several surveys have shown a direct correlation between serum retinol (SR) and Hb levels in women and children (Mohanram et al., 1977; Suharno et al., 1993; Woldegebriel et al., 1993) and vitamin A supplementation in populations with low SR levels resulted in increased Hb concentrations (Mejia & Arroyave, 1982; Mejia & Chew, 1988; Muhilal et al., 1988b), suggesting that vitamin A deficiency impairs iron mobilization form stores and has little influence on Fe absorption (Lynch, 1997; Allen & Casterline-Sabel, 2001). Supplementation with riboflavin caused an increase of Hb concentration in riboflavin deficient individuals (Powers et al., 1983b; Powers et al., 1985a) and Hb levels were increased more when Fe and riboflavin were given together than with Fe alone (Decker et al., 1977; Buzina et al., 1979). Suggested mechanisms are increased intestinal loss, reduced absorption, impaired mobilization of intracellular Fe, increased crypt cell proliferation and impaired synthesis of globin and reduced activity of NADH-FMN oxidoreductase so that Fe becomes trapped in ferritin (Bates, 1987; Powers et al., 1991; Fairweather-Tait et al., 1992; Allen & Casterline-Sabel, 2001).

Iodine

Iodine is a constituent of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) and therefore is an essential micronutrient for all animal species, including humans (Hetzel, 1993). These hormones are essential for normal growth and physical and mental development. Iodine occurs in foods mainly as inorganic iodide, which is readily and almost completely absorbed from the gastrointestinal tract and absorption appears not to be influenced by other food components (Keating & Albert, 1949). Necessary amounts of iodide are removed from the circulation by the thyroid for hormone synthesis, and the remainder is excreted by the kidney (Hetzel, 1993). The thyroid secretion is under the control of the pituitary gland through the thyroid stimulation hormone (TSH), which increases when T4 falls and results in an increase of thyroid activity (Hurrell, 1997a). To ensure adequate supply of thyroid hormones a
daily allowance of 150 µg iodine is recommended for adolescents and adults (Institute of Medicine, 2001).

In populations living in areas where soil has a low iodine content as a results of past glaciation or the repeated leaching effects of snow, water and heavy rainfall crops grown on this soil do not provide adequate amounts of iodine (Hetzel et al., 1987; Hetzel, 1993; Delange, 1994). The thyroid is able to adaptively respond to a certain extent to iodine deficiency throughout its metabolic pathway by increasing iodine uptake into the thyroid, maximizing hormone production per atom iodine, rapid turnover of iodine, recycling of all iodine not secreted as hormone and by increasing of the thyroid mass (Dunn, 1996). When the thyroid is no longer able to synthesize sufficient amounts of thyroid hormones, plasma levels of T4 and T3 decrease (hypothyroidism) and result in harmful effects on growth and development, collectively known as Iodine Deficiency Disorders (IDD) (Hetzel, 1983). Iodine deficiency of the fetus due to maternal iodine deficiency is most serious and leads to a greater incidence of stillbirths, spontaneous abortions, congenital abnormalities and cretinism and can be reduced by iodization (Hetzel, 1993). There are two types of endemic cretinism: the most common neurological form, which is characterized by mental deficiency, deaf mutism and spastic diplegia and the less common hypothyroid type characterized by thyroid failure and dwarfism. In the neonate, iodine deficiency leads to increased perinatal and infant mortality (Thilly, 1981), whereas in adults and children it is normally associated with goiter but it also leads to hypothyroidism with a lower metabolic rate and impaired mental function (Hetzel, 1993). IDD is still a public health problem affecting 35% of the world population corresponding to nearly two billion individuals (WHO, 2004). The most affected region is South-East Asia followed by Africa and the Western Pacific. Since iodine deficiency is the single most important preventable cause of brain damage and IDD are among the easiest and cheapest of all disorders to prevent its elimination is a critical development issue (WHO/UNICEF/ICCIDD, 2001; WHO, 2004).

There is evidence that common micronutrient deficiencies in developing countries such as vitamin A, selenium and iron can each exacerbate the effects of iodine deficiency. (Zimmermann et al., 2000b; Zimmermann et al., 2000a; Hess et al., 2002a; Hess et al., 2002b; Hess & Zimmermann, 2004).
Literature Review

Vitamin A

Vitamin A (retinol) is an essential, fat-soluble nutrient needed for the normal functioning of vision, cellular differentiation and proliferation, immune response, hemopoiesis, growth and reproduction (Ross, 1999; FAO/WHO, 2001c; McLaren & Frigg, 2001). Vitamin A is provided as preformed retinol (retinyl esters) of animal origin, and as provitamin A (carotenoids, including β-carotene as the most important) of vegetable origin (IVACG, 1989). The absorption of retinol is 70-90% whereas that of carotenoids is only 20-50% (Sivakumar & Reddy, 1972) and absorption is influenced by several factors including dietary fat content, intestinal parasite infection and diarrhea (Mahalanabis et al., 1979; Reddy et al., 1986).

Vitamin A deficiency (VAD) was defined by WHO as tissue concentrations of vitamin A low enough to have adverse health consequences, which are in addition to the specific clinical signs of xerophthalmia and the risk of irreversible blindness, non-specific symptoms including increased morbidity and mortality, poor reproductive health, increased risk of anemia and contributions to slowed growth and development (WHO, 1996). More than 100 million children in the world are vitamin A deficient with the highest prevalence in Africa and South-East Asia (WHO, 2003b) with a population rarely consuming animal products resulting in vitamin A intakes mainly from vegetable sources containing the poorly bioavailable carotenoids (FAO/WHO, 2001c).

There is evidence that VAD can affect iron metabolism in iron deficient individuals (IVACG, 1998b) and several vitamin A supplementation or fortification trials have shown an increase in Hb (Mejia & Chew, 1988; Muhilal et al., 1988b; Suharno et al., 1993; Mwanri et al., 2000) or an improvement in iron status indicators (Bloem et al., 1990; Semba et al., 1992). It appears that VAD influences the availability of storage iron for use by hematopoietic tissue (Sijtsma et al., 1993; Suharno et al., 1993). However, a study looking at vitamin A and iodine interaction suggests, that mild-to-moderate VAD in IDD-affected children decreases the risk for hypothyroidism (Zimmermann et al., 2004c).

Riboflavin

Riboflavin, also called vitamin B₂, is a water soluble, heat stable and light sensitive vitamin and functions as precursor of the flavin coenzyme in numerous oxidation-
reduction reactions in metabolic pathways and in energy production via the respiratory chain (McCormick, 1997).

Riboflavin deficiency is endemic in many developing countries where people consume diets lacking in dairy products and meat (Bates et al., 1982; Powers et al., 1985a; Wilson, 1988; Neumann et al., 2003) and causes a variety of symptoms including impaired growth, cheilosis, angular stomatitis, glossitis and dermatitis (McCormick, 1994).

There is good evidence that poor riboflavin status interferes with iron handling and contributes to the etiology of anemia when iron intakes are low. Several studies in pregnant or lactating women, adult males, and school children have shown improved hematological response to iron supplements when riboflavin deficiency was corrected (Decker et al., 1977; Buzina et al., 1979; Powers et al., 1983b; Powers et al., 1985b).

Proposed mechanisms from animal studies are reduced ferritin iron mobilization through a lack of reduced flavins (Sirivech et al., 1974; Crichton et al., 1975; Sirivech et al., 1977; Powers et al., 1983a; Powers, 1987), impaired iron absorption and an increased rate of gastrointestinal Fe loss due to morphological changes in the small intestine (Powers et al., 1988; Powers et al., 1991; Powers, 1995). Riboflavin also plays a role in thyroxine metabolism, and riboflavin deficiency may contribute to the pathophysiology of some mental illness via this route (Bell et al., 1992).

Assessment of micronutrient status

Iron

Worldwide, the most common method of screening individuals or populations for iron deficiency is to determine the prevalence of anemia by measuring blood hemoglobin or hematocrit levels (WHO/UNICEF/UNU, 2001). The determination of the prevalence of anemia is relatively simple and inexpensive and the two recommended methods by WHO are the cyanomethemoglobin method in the laboratory (1994) and the HemoCue system in the field (Vonschenck et al., 1986). Hematocrit measurement is an acceptable and recommended method for anemia determination, but has no advantage compared to hemoglobin measurement (WHO/UNICEF/UNU, 2001). However, anemia is not a specific indication for ID and can result from other nutrient deficiencies and infectious diseases which limits the application of these two tests (WHO/UNICEF/UNU, 2001).
A series of further, specific, and more sensitive tests can be used to characterize Fe status precisely and to categorize the severity of ID (Institute of Medicine, 2001; WHO/UNICEF/UNU, 2001). Unfortunately, there is no single standard test to assess ID without anemia and the use of multiple tests only partially overcomes the limitation of a single test (Dallman et al., 1981) and is not an option in resource-poor settings due to their relatively high cost (WHO/UNICEF/UNU, 2001).

To determine depletion of Fe stores serum ferritin concentration (SF), total iron binding capacity (TIBC) or bone marrow Fe stain can be measured (Institute of Medicine, 2001). The concentration of SF is proportional to the size of body Fe stores in healthy individuals and those with early ID and indicates total depletion of Fe stores when it falls below 15 µg/L for individuals > 5 y and below 12 µg/L for those younger than 5 y (WHO/UNICEF/UNU, 2001). SF is a universally available and well standardized measurement that has been the single most important and recommended laboratory measure of Fe status during the past quarter century (Beutler et al., 2003). However, the well known limitation of the SF which is an acute-phase reactant protein is the elevation in values independent of Fe status that occur with acute or chronic inflammation and infection (WHO/UNICEF/UNU, 2001; Beutler et al., 2003). TIBC is not a useful indicator due to diurnal variation, a marked overlap between normal and iron-deficient subjects and the influence of infection (Institute of Medicine, 2001; WHO/UNICEF/UNU, 2001). Although bone marrow iron-staining is not useful in simple population-based surveys it still is regarded as the gold standard against which Fe tests are evaluated (WHO/UNICEF/UNU, 2001).

Early functional Fe deficiency can be determined by measuring serum transferrin saturation, free erythrocyte or zinc protoporphyrin and serum transferrin receptor (TfR) (Institute of Medicine, 2001). Serum transferrin saturation decreases as the iron supply decreases and levels below 16% saturation indicate that the rate of delivery of Fe is insufficient to maintain the normal rate of hemoglobin synthesis (Institute of Medicine, 2001). However, low saturation levels are not specific for ID and are encountered in other conditions such as anemia of chronic disease (Cook, 1999). Levels of erythrocyte protoporphyrin, the precursor of heme, become elevated (cutoff: >70 mmol/mol heme) when the Fe supply is inadequate for Hb synthesis (WHO/UNICEF/UNU, 2001). Similarly, ID leads to an incorporation of zinc into protoporphyrin instead of Fe and measuring zinc protoporphyrin:heme ratio by a hematofluorimeter from a drop of whole blood indicates impaired heme synthesis.
(Beutler et al., 2003). However, infection or inflammation, lead poisoning and hemolytic anemia also cause an elevation in these two indicators (WHO/UNICEF/UNU, 2001; Beutler et al., 2003), although the interference can be eliminated with prior washing of the red cells (Zanella et al., 1993). The measurement of serum TfR is a recent addition to the available selection of tests for ID and major advantages involve the facts that the assay is not significantly affected by infection or inflammatory processes and does not vary with age, gender, or pregnancy (Kohgo et al., 1986; Carriaga et al., 1991). However, recent studies have shown that TfR is not useful in detecting iron deficiency in individuals with asymptomatic malaria because malarial-associated hemolysis may also increase TfR concentration (Mockenhaupt et al., 1999; Stoltzfus et al., 2000; Menendez et al., 2001; Verhoef et al., 2001). An increase in TfR is a sensitive response during the early development of ID since it increases progressively as the supply of Fe to the tissues becomes progressively more deficient (WHO/UNICEF/UNU, 2001). The most commonly used method to measure TfR is based on the ELISA assay (enzyme-linked immunosorbant assay) and its application is limited due to high costs. If an electronic blood counter is available the reduction in mean corpuscular volume (MCV) in parallel with anemia indicates a late phenomenon in the development of ID (WHO/UNICEF/UNU, 2001).

Since each of the above described tests has limitations in terms of its sensitivity and specificity a definition of ID and IDA based on multiple indicators is useful for population-based assessments. The best combination would be Hb, TfR and SF or bone-marrow Fe, which would reflect functional impairment, tissue avidity for Fe, and Fe storage, respectively, although this approach is not feasible in settings with resource constraints (WHO/UNICEF/UNU, 2001).

More recently, a new method for assessing iron status based on the quantitative measurement of body iron has been described (Cook et al., 2003). The method requires the ratio of the two parameters TfR and SF (R/F ratio) for the calculation of body iron in mg using the following formula:

\[
\text{body Fe} = -[\log(R/F \text{ ratio}) - 2.8229]/0.1207
\]

The method has two major advantages. First, body iron can be expressed on the basis of body weight rather than absolute values thereby eliminating the effect of differences in body weight and permitting extrapolation to younger persons. Second, TfR and SF can be determined from a small capillary blood sample. This is a
substantial advantage, particularly in developing countries where getting permission for venous blood sampling is not always easy. The method has been shown to be useful for monitoring iron status in highly susceptible people and for calculating iron absorption in supplementation and fortification trials.

Iodine

There are clinical and biochemical indicators to assess iodine status of a population. Thyroid size, which enlarges in iodine deficient individuals, can be measured by palpation or ultrasonography (WHO/ICCIDD/UNICEF, 1999). In contrast to palpation, ultrasonography provides precise and reliable measurement of thyroid volume and is replacing palpation in most studies. Thyroid size is useful in the baseline assessment of the severity of IDD and for measuring the long-term impact of control programs (WHO/UNICEF/ICCIDD, 2001).

Goiter responds slowly to a change in iodine status and therefore urinary iodine (UI) is today recommended as a more sensitive indicator of recent changes in iodine nutrition since most iodine absorbed in the body eventually appears in the urine (WHO/UNICEF/ICCIDD, 2001; WHO, 2004). Many analytical techniques exist, varying from very precise to semi-quantitative low tech methods. Most methods depend on iodide’s role as catalyst in the reduction of ceric ammonium sulfate (yellow color) to the cerous form (colorless) in the presence of arsenious acid after digestion with ammonium persulfate currently recommended by WHO (Pino et al., 1996; WHO/UNICEF/ICCIDD, 2001). Median urinary iodine concentrations of >100 µg/L define a population with adequate iodine intake. Values of < 20 µg/L indicate severe, values between 20-49 µg/L moderate and values between 50-99 µg/L mild iodine deficiency (WHO/UNICEF/ICCIDD, 2001).

Two blood constituents, thyroid stimulating hormone (TSH) and thyroglobulin (Tg) can serve as surveillance indicators (WHO/UNICEF/ICCIDD, 2001). Blood spots on filter paper or serum samples can be used to measure TSH and/or Tg. Since circulating T₄ is generally lowered and serum TSH risen during iodine deficiency median TSH concentration is usually higher in an iodine deficient population. However, the difference is not great unless the iodine deficiency is moderate or severe and TSH concentration is therefore not a practical marker for iodine deficiency in school children and adults but is highly recommended for screening of neonates. The thyroid hyperplasia of iodine deficiency is associated with increased
serum Tg levels and reflects iodine nutrition over a period of months or years. Determining serum concentrations of T₄ and T₃ is usually not recommended for monitoring iodine nutrition because these tests are more cumbersome, more expensive, and less sensitive.

Vitamin A

Several clinical and subclinical indicators exist to measure vitamin A status. Clinically well-established indicators of severe VAD are obvious eye signs of vitamin A deficiency, i.e. Bitot's spot, corneal xerosis, keratomalacia and corneal scars (WHO, 1996). However, this clinical signs of xerophthalmia are inadequate for assessing the prevalence of non-clinically observable deficiencies, such as depletion of vitamin A stores to the level where important functional consequences for health are likely to occur (WHO, 1996).

Subclinical indicators include the functional indicator of night blindness, the biochemical indicators of serum retinol (SR), relative dose response test and total liver reserves by isotope dilution and the histological indicator of impression cytology (WHO, 1996; Institute of Medicine, 2001). SR is the best established biochemical indicator although it reflects body stores only when these are very low or very high and in the absence of infection and protein or energy malnutrition (WHO, 1996; Institute of Medicine, 2001). Although the most expensive method and difficult to maintain under laboratory conditions in developing countries, HPLC is the method of choice for SR due to its high specificity and sensitivity (WHO, 1996; de Pee & Dary, 2002). SR concentrations <0.70 μmol/L indicate low vitamin A status (Arroyave et al., 1989; WHO, 1996; de Pee & Dary, 2002).

Riboflavin

The most common indicators to measure riboflavin status in humans are erythrocyte glutathione reductase activity coefficient (EGRAC), erythrocyte flavin concentration and urinary excretion of the vitamin in fasting, random, or 24-hour specimens or by load tests (Nicholads, 1981; Institute of Medicine, 1998). Currently, ECRAC is the most commonly used method, determining the FAD-dependent glutathione reductase activity in freshly lysed red cells (Sauberlich et al., 1972; McCormick & Green, 1994). Values are expressed as 'activity coefficients' and coefficients <1.2 indicate normal riboflavin status, 1.2-1.4 low status and >1.4 deficiency (McCormick, 1994). The
drawback of this assay is, that it can not be used in people with glucose 6-phosphate deficiency due to increased avidity in the reductase for FAD (Nichoalds, 1981). The method measuring erythrocyte flavin concentration by HPLC after conversion of the instable FAD to the more stable FMN is a useful indicator reflecting the functional, cellularly trapped forms of riboflavin (Institute of Medicine, 1998). Urinary riboflavin is used to estimate riboflavin requirements and is influenced by many factors including sampling time, physical activity, urinary volume, creatinine in the diet, nitrogen balance and stress (Bates, 1997).

Influence of infection on micronutrient status, anemia and the measurement of micronutrient status

Indicators of inflammation

Inflammation is itself a complex series of interacting pathways and cascades that involves many different cell types and mediators (Raynes, 1994). The hepatic plasma proteins, which are induced following inflammation and therefore are a fundamental aspect of many diverse disease processes, are collectively known as acute phase reactants or acute phase proteins (APPs) (Pepys & Baltz, 1983; Raynes, 1994). There are two types of APPs classified on the basis of how they change during an acute phase response: those that increase are positive APPs (C-reactive protein (CRP), α1-acid glycoprotein (AGP), α1-antichymotrypsin (ACT), serum ferritin) and those that decrease negative APPs (transferrin, albumin, transthyretin, retinol binding protein (RBP)) (Koj, 1985). CRP is very sensitive and plasma concentrations increase within 10 h of the onset of acute inflammation and normalize rapidly, usually within 1 wk (Louw et al., 1992). However, slight elevations can also occur during low grade chronic inflammation (Thompson et al., 1995). ACT concentrations also increase early, but remain elevated for a longer time than CRP. In contrast, AGP concentrations begin to increase only >24 h after the onset of inflammation, but remain elevated well into convalescence and concentrations are also elevated in low grade chronic inflammation (Fleck & Myers, 1985; Louw et al., 1992). Since during the acute phase response plasma concentrations of many micronutrients change measuring an APP could help to interpret data on micronutrient status (Thurnham, 1997). Several studies have shown that malaria and chronic worm infections are
related to acute phase markers (Mansor et al., 1991; Graninger et al., 1992; Filteau et al., 1993; Ghosh et al., 1995) and WHO recommends to measure one or more APPs, including the most frequently used CRP, ACT, AGP, serum amyloid A, fibrinogen and haptoglobin to assess iron status of populations (WHO/CDC, 2004). The authors state that AGP maybe is a better indicator of the presence of chronic, sub-clinical infections than CRP or ACT.

Malaria

Malaria causes 1.3 million deaths each year worldwide and 90% of the deaths are in sub-Saharan Africa, predominantly in children < 5 y (Bates et al., 2004; Lee, 2004). In areas of stable malaria transmission, very young children and pregnant women are the population groups at highest risk for malaria morbidity and mortality (WHO & UNICEF, 2003). Although nutritional deficiencies, hookworm infection, and HIV all predispose to anemia in children, evidence suggests that, in endemic countries, malaria is one of the most important contributory factors (WHO, 2002) and is estimated to cause about two-third of cases of anemia in infants and children (Sharp & Harvey, 1980; Menendez et al., 1997).

Malarial anemia is believed to be the result of impaired erythropoiesis and probably is exacerbated by malaria-induced hemolysis (Menendez et al., 2000). Malarial anemia is associated with a shift of iron distribution from functional compartments – comprising metabolically active iron that is required for normal functions – toward storage compartments that constitute an iron reserve (Das et al., 1997), which suggests a relative deficit in erythropoietin production or decreased marrow responsiveness to erythropoietin in malaria, similar to that observed in other infections (Means & Krantz, 1992). Studies have shown reduced numbers of red cell precursors and reduced incorporation of Fe into red blood cells during acute malaria (Srichaikul et al., 1969; Abdalla, 1990). Antimalarial treatment of infants in Tanzania not only resulted in a reduction of episodes of clinical malaria but also in a 50% reduction of episodes of severe anemia (Schellenberg et al., 2001). One study has shown that even asymptomatic malaria results in decreased Hb concentrations and that severe anemia can develop following prolonged exposure to chronic or repeated infection with low levels of parasitemia (Verhoef et al., 2002).

Malaria has been shown to influence vitamin A status resulting in a reduction of SR levels characterized as a direct consequence of the inflammatory response to
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*Plasmodium* infections (Thurnham & Singkamani, 1991; Tabone et al., 1992; Friis et al., 1997; Thurnham, 1997; Hautvast et al., 1998).

Looking for markers of inflammation in children with severe malarial anemia, Biemba et al. (2000) found that serum neopterin concentrations were higher in cerebral malaria children with severe anemia compared to cerebral malaria children with Hb concentrations ≥ 70 g/L. The influence of malaria on the most commonly measured acute phase protein CRP is contradictory. A study in pregnant Tanzanian women has shown no correlation of CRP levels with malaria (Hinderaker et al., 2002) and a study comparing CRP values of patients with acute malaria with patients with other febrile illnesses showed no difference (Eriksson et al., 1989). Another study in children has shown that malaria-attributable increased CRP levels are highly dependent on age, with a higher attribution in infants <1 y than in older children (Hurt et al., 1994b). However, several studies in infants, children and adults have shown elevated CRP concentrations during afebrile and acute malaria (Naik & Voller, 1984; Gillespie et al., 1991; Graninger et al., 1992; Hurt et al., 1994a; McGuire et al., 1996; Hautvast et al., 1998). AGP has been shown to be associated with malaria in children and maybe is a better indicator to correct for micronutrient status measurements than CRP since it probably better detects asymptomatic malaria cases in semi-immune individuals (Mansor et al., 1991; van Hensbroek et al., 1996; Hautvast et al., 1998).

**Helminths and Schistosomiasis**

Iron deficiency anemia is a major public health problem for people living in developing countries and its cause is usually complex and multifactorial but several types of parasitic worms can make a significant contribution, usually by causing a loss of blood (Hall et al., 2001). Around 2 billion people (1/3 of the world's population) are infected with schistosomes and soil-transmitted helminths and at least 50% of the 300 million severely ill individuals are school-age children (WHO, 2003c). Infections due to hookworms (*Necator americanus* and *Ancylostoma duodenale*) are almost ubiquitous over large areas of the tropics and subtropics and persistently highly prevalent (Pawlowski et al., 1991).

Adult hookworms live in the duodenum and jejunum from which they suck blood (Pawlowski et al., 1991). The most serious consequence is chronic blood loss leading to IDA (Pawlowski et al., 1991). Several mechanisms are thought to contribute to such anemia: the feeding on blood of the worms (Pawlowski et al.,
continuing bleeding from sites where the worms were attached as a result of the secreted anticoagulants (Kalkofen, 1974), impaired Fe uptake of the host due to the presence of worms at the same site of Fe absorption (Bothwell, 1996) and probably impaired appetite resulting in poor Fe intake (Hadju et al., 1996).

A heavy infection of the ubiquitous species *Trichuris trichiura* can also produce anemia resulting from blood loss due to dysentery and damage to the mucosal epithelium (Bundy & Cooper, 1989).

An association between *Ascaris lumbricoides* and anemia has been observed in school age children in Nepal (Curtale et al., 1993). The suggested mechanism is malabsorption of iron because the worm lives in the duodenum and jejunum the sites where iron absorption occurs (Bothwell, 1996). Another study in Nepal showed that the presence of *A. lumbricoides* eggs was associated with lower levels of SR in children <5 y of age and that children receiving mebendazole had significantly higher levels of SR (Curtale et al., 1994).

Three major species of trematode blood flukes of the genus *Schistosoma* living in the blood vessels around the intestine (*S. mansoni, S. japonicum*) or in the blood vessels around the bladder (*S. haematobium*) are associated with blood loss (Sturrock, 1993; Hall et al., 2001). This blood loss is largely due to the parasite's eggs which from the blood vessels penetrate the tissue to get to the gut or bladder lumen and cause bleeding at the site of penetration. (Sturrock, 1993). Several studies have shown an association between Hb concentration and intensity of infection with *S. haematobium* and *S. mansoni* (Stephenson et al., 1985; Prual et al., 1992; Sturrock et al., 1996). A study from Kenya has shown that *S. mansoni* egg output was a predictor of low SR in school children and could be due to an effect of infection on SR or an increased susceptibility to infection among children with low SR (Friis et al., 1997). This relation was not observed in preschool children.

**Influence of infection on iron metabolism**

It is since long known that iron metabolism is altered during infection and inflammation and can result in the anemia of chronic disease (ACD). In ACD circulating iron levels are low, iron stores are adequate or increased and intestinal iron absorption is decreased (Lee, 1983). ACD is generally normocytic, but may be microcytic when the total body iron content is low. The pathogenesis of ACD is not
fully understood, but poor maintenance of red blood cell mass has been observed at three levels (Roy et al., 2003).

First, and maybe most important, the normal flow of iron between tissues is altered in ACD. In the normal flow iron for erythropoiesis is provided by transferrin (Cartwright, 1966). ACD patients have significantly decreased transferrin saturations and consequently red blood cell mass decreases. Clinical efforts to elevate serum iron and improve erythropoiesis often fail. However, the lack of response to iron is not caused by impaired erythroid acquisition of iron, because iron given in the form of diferric transferrin is rapidly taken up by erythroid precursor cells suggesting that the transfer of iron from macrophages to transferrin is defective in ACD. The second observation in ACD patients is that erythroid precursors respond poorly to erythropoietin, the growth factor necessary for red blood cell differentiation and Hb synthesis (Roy et al., 2003). The blunted erythropoietin response has been described to be due to inflammation- or cytokine-induced changes in erythroid precursors. In addition to the poor response of erythroid precursors to erythropoietin, individuals with ACD have also been observed to indeed have increased erythropoietin levels but not to the same extent as is seen in patients with similar degrees of anemia resulting from iron deficiency alone (Means, 2000). Third, the observed lifespan of circulating red blood cells in individuals with ACD is decreased, apparently due to more avid phagocytosis by cells of the reticuloendothelial system (Cartwright, 1966). This increased avidity of reticuloendothelial macrophages in ACD may represents an attempt to increase the amount of iron available to developing red blood cells.

More recently, a new gene came into the discussion of the etiology of ACD. Several studies have presented evidence that inappropriate expression of hepcidin is associated with severe ACD (Roy et al., 2003). Hepcidin is an antibacterial protein produced in the liver which can be found in blood or urine, and which participate in host defense functioning as an acute-phase protein (Park et al., 2001). In terms of its role in physiologic iron balance, it appears to enhance iron uptake and retention by reticuloendothelial cells in the duodenal crypts, while decreasing dietary iron absorption (Fleming & Sly, 2001). A recent study suggests that the major mechanism of hepcidin function seems to be the regulation of transmembrane iron transport (Vyoral & Petrak, 2005). The transport of iron across enterocyte basolateral membrane is mediated by a membrane transporter ferroportin1 and iron finally appears in circulation bound to transferrin (Frazer & Anderson, 2003).
of iron efflux form enterocytes (and also from haptocytes and macrophages) is mediated by a newly discovered peptide hepcidin. Lysozomal degradation of ferroportin induced by circulating hepcidin results in trapping of iron in haptocytes, macrophages and absorptive enterocytes. As a result, iron absorption and mobilization of storage iron from liver and macrophages is lowered (Knutson et al., 2005), causing a decrease of available serum iron. Elevated hepcidin concentration during inflammation and infection (Ganz, 2003) leads to a decreased blood iron concentration and thereby to a diminished availability of iron for invading microorganisms. However in the long term, this hepcidin induced decrease of serum iron results in anemia of chronic disease (Andrews, 2004).

**Influence of infection on micronutrient status indicators**

Since during the acute phase response plasma concentrations of many micronutrients change (Thurnham, 1997) the prevalence of micronutrient deficiencies assessed by commonly used indicators in people living in areas with endemic malaria and chronic infections often are overestimated (vitamin A, zinc) or underestimated (IDA) (Wieringa et al., 2002). This may result from a redistribution of the indicators of micronutrient status during infection to protect the host or his micronutrient reserves in the short term without a real change in the total body content of the micronutrient (Thurnham, 1997; Beisel, 1998).

In order to withhold growth essential Fe from pathogens, Fe is taken out of the circulation and stored in intracellular ferritin as a host defense reaction, thus resulting in the anemia of infection and chronic disease with subnormal serum Fe, hemoglobin, and transferrin saturation values but normal to elevated SF values (Kent et al., 1994). In addition SF is an APP which increases during inflammation and malaria resulting in a underestimation of the prevalence of ID and IDA (Wieringa et al., 2002). TfR has the advantage not to be influenced by infection and inflammation (Oppenheimer et al., 1984; Skikne et al., 1990; Ferguson et al., 1992; Nielsen et al., 1994; Ahluwalia et al., 1995; Kuvibidila et al., 1995; Suominen et al., 1997; Staubli Asobayire et al., 2001) but recent studies have shown that TfR is not useful in detecting iron deficiency in individuals with asymptomatic malaria because malarial-associated hemolysis may also increase TfR concentration (Mockenhaupt et al., 1999; Stoltzfus et al., 2000; Menendez et al., 2001; Verhoeef et al., 2001). However, other studies have found no significant difference in TfR concentration in pregnant
women and children comparing malaria infected to malaria free individuals (Huddle et al., 1999; Williams et al., 1999; Staubli Asobayire et al., 2001). CRP and AGP correlate with SF (Wieringa et al., 2002) and exclusion of individuals with elevated APPs may help to interpret SF values. However, exclusion of subjects with an acute phase response has the disadvantage of reducing the use of valuable data and numbers of excluded subjects would be very high in tropical countries with malaria and chronic infections. A correction factor, based on effect sizes such as reported by Wieringa et al. (2002) would provide a more elegant solution.

Retinol concentrations are well known to be influenced by disease (Louw et al., 1992; Thurnham, 1997). Since serum levels of circulating retinol are controlled by the level of RBP (Kanai et al., 1968) and RBP is a negative APP (Rosales et al., 1996), concentrations of serum retinol fall in disease. A decline in circulating retinol can result in a serious problem in not well-nourished persons at the outset of disease and if disease becomes chronic which often is the case in developing countries (Thurnham, 1997). Several APPs such as AGP, CRP and amyloid A correlate with SR concentrations and may be used to interpret SR values (Filteau et al., 1993; Wieringa et al., 2002).

An apparent increase in riboflavin status can be observed during infection if measured by the EGRAC method resulting from a release of tissue co-enzymes into the circulation during tissue breakdown and negative N balance (Bates et al., 1981; Bamji et al., 1987).

If thyroid hormones are used as functional markers of iodine status in communities where infection is common, iodine deficiency may be overestimated due to the transport of iodine in the blood by the negative APP transthyretin (Thurnham, 1997). Some studies have shown that infection and APPs (CRP and AGP) are correlated with a fall in serum Zn and Cu concentration resulting in an overestimation of the prevalence of deficiency (Graham et al., 1991; Wieringa et al., 2002). Vitamin C in the blood is strongly influenced by disease and serum ascorbate and leukocyte vitamin C are lowered during infection (Louw et al., 1992; Thurnham, 1997).
Public health strategies

Several public health strategies exist to prevent and combat micronutrient deficiencies, including supplementation to those 'at risk', food-based strategies involving fortification and dietary diversification/modification and biofortification of plant-based staples for those living in subsistence settings (Gibson, 2004).

Supplementation

The ultimate aim of supplementation is to prevent or reduce negative health or developmental consequences of nutrient deficiencies (Ekström, 2001). Supplementation refers to periodic administration of pharmacological preparations of nutrients as capsules or tablets or by injection when substantial or immediate benefits are necessary for the group at risk (L'Abbe et al., 2003). The use of supplements is clearly the most easily utilized approach on an individual basis, but has rarely been used as a population intervention approach and poorer populations are less likely to be reached by supplements than by a food fortification program (L'Abbe et al., 2003). In many developing countries where endemic deficiencies of iodine, vitamin A, and iron exist, oral or intramuscular iodized oil concentrate, high dose vitamin A capsules, and iron pills – delivered annually, biannually, and by daily ingestion – are provided through ongoing, policy-supported programs (Alnwick, 1998).

Intramuscular injection of iodized oil with a protection for 3-5 years has been used successfully for combating goiter and cretinism in hyper-endemic areas (Vijayaraghavan, 1995). However the high cost and the difficulty in reaching all the victims of IDD make this approach less practicable. To date, many countries have reduced their need for iodine supplements by successful universal salt iodization (Underwood & Smitasiri, 1999).

In contrast, large-scale vitamin A supplement distribution continues in many countries with the need for safe distribution by health workers (Underwood, 1990), although more resource-efficient and coverage-effective delivery mechanisms are now used (UNICEF-Manila & Helen Keller International, 1996; Alnwick, 1998). Coverage rates of vitamin A supplementation of young children reached levels up to 90% when a link was made to the campaign approach of the WHO expanded program of immunization (Goodman et al., 2000) and several countries already have established
twice-yearly micronutrient days for the administration of vitamin A capsules (Underwood, 2004). During maternal reproductive processes and lactation needs of vitamin A increase and supplementation in VAD areas is considered as safe and recommended postpartum by the International Vitamin A Consultative Group (IVACG, 1998a; IVACG, 2002).

The provision of iron pills during pregnancy as a strategy for prevention and control of nutritional anemias is the oldest and most common supplementation activity and supplementation policies for pregnant women exist in many countries but are often ineffectively implemented (Underwood & Smitasiri, 1999; Ekström, 2001). Lack of compliance at all levels in the health system as well as by potential beneficiaries, for reasons ranging from adequacy of supply and quality of pills to unpleasant side effects and forgetfulness, accounts for slow progress (Stoltzfus & Dreyfuss, 1998). An additional constraint may arise from cultural barriers when supplement use does not fit in the prevailing sociomedical system and a fear of the women of having too much blood or of making the baby big arose (Ekström, 2001). Beside pregnant women, other target groups to prevent ID are women of childbearing age, infants > 6 months, preschool children and adolescent girls with the problem that all these groups are often difficult to contact through the health services (WHO/UNICEF/UNU, 2001). However, continued dependence on short-term medicinal supplementation programs will not be economical and more cost effective strategies such as dietary diversification are needed in the long run (Vijayaraghavan, 1995).

**Dietary diversification/modification**

Lack of dietary diversity is a particularly severe problem among poor populations from the developing world because their diets are predominantly based on starchy staples and often include little or no animal products and few fresh fruits and vegetables (Ruel, 2003). These plant-based diets tend to be low in a number and bioavailability of micronutrients (Ruel, 2003). Dietary diversification/modification includes a wide variety of interventions that aim to enhance the availability, access, and utilization of foods with a high content and bioavailability of micronutrients throughout the year (Gibson & Hotz, 2001; Ruel & Levin, 2001). It involves changes in food production practices, food selection patterns, and traditional household methods for preparing and processing indigenous foods. This strategy may be more sustainable, economically feasible, and culturally acceptable than supplementation or
Chapter 1

fortification because individuals and households take ultimate responsibility over the quality of their diets and several micronutrient deficiencies can be alleviated simultaneously without risk of antagonistic interactions (FAO/ILSI, 1997; Gibson & Ferguson, 1998).

Home gardens have been by far the most popular food based strategy used to address vitamin A deficiency and resulted in an increase in knowledge, awareness, attitude and consumption of provitamin A rich foods when a strong education and communication component was included (English et al., 1997; Marsh, 1998; Ayalew et al., 1999; Hagenimana et al., 1999; Ruel & Levin, 2001). However, evidence of impact on nutritional status is still scant (Ruel & Levin, 2001).

Interventions for iron are more limited and the situation is more complex due to the high cost of animal foods rich in bioavailable iron (Ruel & Levin, 2001). One problem of animal production is the issue of the trade-offs farmers which tend to increase their income through the sale of produce rather than enriching their diet through consumption of self produced goods as shown in Bangladesh (International Food Policy Research Institute, 1998) and Ethiopia (Ahmed et al., 1999) which demonstrates the importance of education. However, one study from Peru showed a reduction in anemia prevalence among women of reproductive age after consuming foods rich in iron and vitamin C (Carrasco-Sanez et al., 1998).

Another strategy are the various home processing techniques available to either increase the bioavailability of micronutrients or to ensure their retention during preparation, cooking, or other processing techniques including home preservation techniques to increase the availability of the highly seasonal fruits and vegetables rich in provitamin A throughout the year, fermentation or germination to reduce phytate content and thereby increase non-heme iron absorption, food-to-food fortification which could result in an increase of the amount of enhancers and in a decrease in the amount of inhibitors and cooking in iron pots (Ruel & Levin, 2001).

Whatever strategy is applied, to be successful, it must be economically feasible, culturally acceptable and sustainable, and must not increase the cost, preparation and cooking time of the meals or the workload of the caregivers (Gibson & Hotz, 2001).
Biofortification

Biofortification is the process of breeding food crops that are rich in bioavailable micronutrients (HarvestPlus, 2005). Genetic modification can be achieved by two fundamentally different approaches (Lonnerdal, 2003). First, by using conventional breeding and selection techniques, cultivars with the highest content of trace elements, the highest concentration of enhancers of trace element bioavailability and/or the lowest content of inhibitors of trace element absorption can be bred into stable and high-producing lines. Second, genetic engineering techniques can be used to create novel cultivars with the desired properties.

Since staple crops are often the only foods regularly eaten in relatively large quantities in developing countries the optimal food vehicle for biofortification are the major food crops (e.g., rice, wheat, maize, beans, cassava) (Bouis, 2002; Welch, 2002). Thousands of different genotypes of these crops exist with highly varying nutrient content as shown for Zn, Fe and β-carotene (Gregorio, 2002). Comparing the bioavailability of a Fe- and Zn-rich genotype to a lower density genotype in humans showed no improvement in the amount of Fe absorbed but a 40% increase in Zn absorption (King et al., 2000). Several genotypes with low phytic acid content have been identified and tortillas and maize made with low phytic acid maize showed an increase in Fe bioavailability (Mendoza et al., 1998) and Zn absorption (Adams et al., 2000), respectively, compared with wild-type maize.

Fe bioavailability in plant foods could be increased through several genetic engineering approaches. Human lactoferrin and the gene for soybean ferritin have been inserted to rice (Goto et al., 1999; Nandi et al., 2002; Suzuki et al., 2003). However, although the bioavailability of iron from lactoferrin has been shown to be about the same as from ferrous sulfate (Davidson et al., 1990), the bioavailability from ferritin is controversial (Hunt, 2005) and the quantity of iron absorbed from these sources can be expected to only be a valuable addition for infants and young children having low total iron requirements (Lonnerdal, 2003). Potential concerns with high-level expression of ferritin in rice include the color of the rice, which may affect consumer attitude and allergenicity (Lonnerdal, 2003). Another approach was the insertion of a fungal heat stable phytase gene into rice (Lucca et al., 2002). Although high expression levels were achieved, boiling the rice destroyed the phytase activity resulting in a very modest reduction of the phytate content. Another option would be
to introduce Fe absorption enhancers such as ascorbic acid, cysteine-containing peptides or hemoglobin (Fairweather-Tait & Hurrell, 1996; Lucca et al., 2002). Successful introduction of the complete provitamin A (β-carotene) biosynthetic pathway into rice endosperm by genetic engineering has been achieved resulting in the so called golden rice (Beyer et al., 2002). Evaluation of bioavailability and bioefficacy as well as risk assessments are currently carried out.

Biofortification has several advantages: it is cost-effective, due to no costs after development of the plant compared to buying fortificants and production costs during food fortification; it is sustainable, because monitoring costs are low and agricultural productivity of mineral-packed seeds is improved due to increased resistance to disease and other environmental stresses; it could make an impact in relatively remote rural areas where food staples do not enter the marketing system or where processing facilities are relatively small, numerous and widely dispersed (Bouis, 2002).

However, consumer confidence and acceptance of genetically engineered food is limited and before seeds with optimal agronomic, nutritional, and organoleptic qualities are available to farmers, several years and substantial resources will be needed to complete the field work necessary for breeding, propagation, bioavailability, and consumer acceptance (Underwood & Smitasiri, 1999). In the mean time, food fortification as a cost-effective internationally advocated intervention in the less-industrialized world has potential to fill the gap.
FOOD FORTIFICATION

Rationale and objective

Fortification refers to the addition of a single nutrient or a group of micronutrients (the fortificant) to a commonly eaten food (the vehicle) (FAO/WHO, 2001a) usually providing 30-50% of the daily adult requirements at normal consumption of the food vehicle (Venkatesh Mannar & Sankar, 2004). Food fortification has been a successful sustainable public health strategy used for over eight decades in industrialized countries (Mertz, 1997; Venkatesh Mannar & Sankar, 2004) and to date is strongly advocated internationally as a cost-effective intervention for the less-industrialized world (Underwood & Smitasiri, 1999). It can be employed for the following purposes:

To correct or prevent a demonstrated dietary deficiency of those nutrient(s) that are added; to restore nutrients initially present in significant amounts in a food but lost as a result of food processing; to increase the nutritional quality of manufactured food products that are used as the sole source of nourishment, e.g., infant formulas of formulated liquid diets and weaning food; and to ensure nutritional equivalency of manufactured food products substituting other foods, e.g., fortified margarine as a substitute for butter (Lofti et al., 1996). Fortification of food with micronutrients is a valid technology within a food-based approach, where existing food supplies and limited access fail to provide adequate levels of the respective nutrients in the diet like in developing countries and is a supportive link to sustainable long-term dietary change in populations (Tontisirin et al., 2002).

To develop a food fortification program several important steps have been identified (Lofti et al., 1996) including the determination of the micronutrient deficiency prevalence and the micronutrient intake and availability from the typical diet among the population; the estimation of consumption data for potential vehicles and their processing industry chain; the choice of the type and amount of the fortificants; the development of the fortification technology; the determination of interactions, potency, stability, storage, and organoleptic quality and bioavailability as well as effectiveness of the fortified food; the search of government and industry support and development of legislation and regulation for mandatory compliance and quality control as well as campaigns to improve consumer acceptance.
Fortification has the advantage of requiring relatively less change in consumer behavior and food habits than other interventions, such as supplementation or diversification, although this approach does not mean that nutrition education and social marketing can be ignored (Darnton-Hill & Nalubola, 2002). To be sustainable, consumers and policy makers need to be convinced about the needs and benefits of fortification (Darnton-Hill & Nalubola, 2002) and to be effective the fortification process requires that only the fortified food is available in the marketplace (Nantel & Tontisirin, 2002). A frequent problem with fortified food, however, is that some target populations, particularly those located far from urban areas, do not have access to centrally-processed fortified foods (Tontisirin et al., 2002) and that fortification increases product price, although this is only marginally (Underwood & Smitasiri, 1999). Food regulations that clearly identify the foods to be fortified, the type and levels of the fortificant, labeling requirements, quality standards, safety and finally a surveillance program are needed (Tontisirin et al., 2002).

**Food vehicles and fortificants**

The selection of an appropriate food vehicle is very important. The selected food vehicle should be easily accessible and a specified amount of it should be regularly consumed in the local diet (FAO/WHO, 2001b). Other major requirements are centralized processing in a facility that will permit the controlled addition of the fortificant using a simple low-cost technology and stability in appearance, feel, taste and smell in the final fortified food (Lofti et al., 1996; Walter et al., 2001). Since rice, corn, and wheat and their derivatives like flours and meals are the most widely consumed staple foods in many developing countries they are the preferred vehicles for fortification (Walter et al., 2001). All cereals are processed or milled before they are consumed which results in a substantial loss of several micronutrients present in the aleurone layer, e.g. about a two third reduction in Fe and Zn content, but which can be overcome by enrichment (restoring lost nutrients) or fortification (Walter et al., 2001). Other vehicles used for food fortification, although less often are condiments such as fish and soy sauce, curry powder, sugar and salt and vegetable oil, which are particularly important in many countries in Africa and Asia where the staple food is processed either at home or in a small village facility in which fortification is likely to be extremely difficult (Dillon, 2000).
General criteria for selection of fortificants are: good bioavailability during normal shelf life of the fortified product; acceptable color, solubility and particle size as well as no interaction with flavor or color systems; affordable cost; free commercial availability of food grade material and in encapsulated form if required and feasibility of addition and dispersion through dry blending or spray coating using premixes if required (Lofti et al., 1996; FAO/WHO, 2001b).

Application and experience

Starting in the 20th century, fortification was used to target specific health conditions in the developed world: goiter with iodized salt; rickets with vitamin D-fortified milk; beriberi, pellagra an anemia with B-vitamins and Fe-enriched cereals; risk of pregnancy affected by neural-tube defects with folic acid-fortified cereals (Darnton-Hill & Nalubola, 2002). A relative lack of appropriate centrally-processed food vehicles, less-developed commercial markets and relatively low consumer awareness and demand has meant that another 50 years passed before fortification was seen as a viable option for the less-developed and industrializing countries (Darnton-Hill & Nalubola, 2002). In the proposed nutrition goals of the UN General Assembly's Special Session on Children in May 2002 fortification is specifically mentioned as an intervention strategy to eliminate IDD by 2005, and VAD by 2010, and to reduce by one third the prevalence of anemia, including ID by 2010 and other micronutrient deficiencies (UN, 2002). Experience from Latin America with vitamin A in sugar and Fe and B-vitamins in cereals indicates the potential for considerable expansion of fortification as an approach to address micronutrient malnutrition in the developing world (Murphy, 1996).

Iodine

The most common food-fortification practice has been salt iodization, which has been in existence for over 80 years. It was first introduced in the 1920s in the United states (Marine & Kimball, 1920) and Switzerland where it resulted in a gradual eradication of both cretinism and goiter (Burgi et al., 1990). However, this strategy was not widely replicated until the 1990s when the World Health Assembly adopted universal salt iodization (USI) for both human and livestock consumption as the method of choice to eliminate IDD (WHO, 2004). USI was chosen as the best strategy because salt is one of the few commodities consumed by everyone at a fairly stable level throughout
the year, it is usually produced centralized in few factories, the iodization technology is easy to implement and available at a reasonable cost (0.4 – 0.5 US cents/kg, or 2 – 9 US cents per person/year), the quality of iodized salt can be monitored at the production, retail and household levels and of special importance the addition of iodine to salt does not affect its color, taste or odor (WHO, 2004). The fortificants commonly used are potassium iodide (KI) and potassium iodate (KIO₃) with the latter being recommended in tropical climates due to its greater stability (WHO et al., 1996). In order to provide 150 μg/day of iodine via iodized salt it is recommended to add 20 – 40 mg iodine per kg of salt at the production site assuming an average salt intake of 10 g per capita/day and taking into account a 20% loss of iodine from production site to household and another 20% loss during cooking (WHO et al., 1996). However, when the salt quality is poor, or the salt is incorrectly packaged, or the salt deteriorates due to excessive long-term exposure to moisture, heat, and contaminants often observed in tropical climates, iodine losses from production to consumption can be well in excess of 50% (WHO et al., 1996). In addition, salt consumption is sometimes considerably less than 10g/person/day (Hess et al., 1999). The iodine concentration of salt needs to be monitored regularly at a minimum at the factory and household levels, and if possible at the retail level and if imported at the point of entry into the country to ensure that the salt industry complies with the regulations set by the government and that the iodine levels are re-adjusted if necessary (WHO, 2004). The iodine content of salt is best measured by titration, although field test kits have been developed to give qualitative results, indicating if iodine is present or not (WHO/UNICEF/ICCIDD, 2001).

Today salt iodization is the example of successful large-scale fortification in the developing world due to the simple and low-cost technology and the narrow band of salt consumption quantities with a given region or population (Venkatesh Mannar & Sankar, 2004). But also international endorsement and advocacy and the coalition of partners were important steps (Darnton-Hill & Nalubola, 2002). By the year 2002, more than 170 countries had committed themselves to universal iodization of salt and today, nearly 70% of the world’s human and livestock salt is iodized (Holland, 2004). However, although USI may be in reach, we are not yet there: in as many as 50 countries less than 50% use iodized salt and a disturbing sign of “backsliding” has been recently noted in some regions (Holland, 2004). There is an urgent need to
reinvigorate the global effort and to accelerate country level action in order to achieve the goal of eliminating IDD, originally set at the year 2005, as soon as possible.

**Vitamin A**

Identifying condiments or single foods with the characteristics necessary for mandatory vitamin A fortification comparable to that of salt in the case of iodization are less easy. However, sugar has been fortified with retinyl palmitate on a national scale in Guatemala since 1974 and although it was suspended various times this program has virtually eliminated VAD as a public health problem over the last 10 years (Arroyave et al., 1979; Krause et al., 1998). Other Central American countries, including Costa Rica, Honduras and El Salvador have followed and the programs are estimated to reach about 95% of households in El Salvador and Guatemala, and more than 80% in Honduras and have significantly reduced the prevalence of low SR levels (Mora et al., 2000). In 1998 Zambia initiated and is ongoing sugar fortification despite constraints like the falling international price of sugar and the continuous infiltration of cheaper unfortified sugar from bordering countries (Darnton-Hill & Nalubola, 2002). Other African and Asian countries are also exploring the feasibility of sugar fortification with vitamin A (Micronutrient Initiative et al., 1999) and in Uganda and three West African countries sugar was identified as a feasible vehicle (Bégin & Greig, 2002; Kawuma, 2002).

Cooking fats and oils offer an option to deliver a portion of fat soluble vitamins such as vitamin A and have the advantage that they are often centrally refined and packaged (Venkatesh Mannar & Sankar, 2004). Furthermore, oil stabilizes retinol and delays oxidation of the vitamin (Johnson, 1997). Retinyl palmitate has been used to fortify refined soybean oil in Brazil, and studies have shown that it remains stable during commonly used storage and cooking procedures (Desal et al., 1991) and that it retains 100% of its biological value after cooking and 50% after repeated deep frying in rats (Favaro et al., 1992). A human study has shown that the absorption of β-carotene from heated or unheated fortified soybean oil is similar (Dutra-de-Oliveira et al., 1998). After oil, margarine and other hydrogenated oil products are the most suitable vehicles for vitamin A and the fortification of margarine was initiated in Denmark in the 1920s to imitate the nutritional value of butter (Bloch, 1931). Several countries fortify margarine at levels ranging from 1 to 15 mg/kg as either a mandatory or a voluntary practice (Johnson, 1997). In the Philippines, a margarine made from a
blend of coconut oil and hardened palm oil was very efficacious in decreasing the prevalence of low SR in preschool-aged children and is now offered in the free market along with competing brands (Solon, 1997a). Vanaspati, a fortified hydrogenated oil, has been available since 1953 in India and is replacing the traditional ghee (Sridhar, 1997).

Fortification of cereal flours with vitamin A is technically feasible, and nutrient stability in the products is good (Dary & Mora, 2002). Venezuela has had a national program fortifying precooked maize flour since 1993 but biological evidence of the impact has not been published (Chavez, 1997). Fortification of wheat flour with vitamin A resulted in a significant increase in vitamin A liver stores of school children (Solon, 1997b).

Monosodium glutamate (MSG) has been fortified with retinyl palmitate and demonstrated efficacy in the Philippines and Indonesia (Solon et al., 1985; Muhilal et al., 1988a). However, problems of discoloration of the white MSG and the separation of the vitamin A compound was unacceptable, particularly in large packs (Solon et al., 1985). Some of the technological problems were overcome in Indonesia by reducing the vitamin A level resulting in a low provision of ≈ 30% of the RDA for vitamin A (Muhilal et al., 1988a). However, increment in the price due to fortification is rather high with 11.5%.

Technological and feasibility issues have prevented large-scale implementation of the vitamin A fortification of other vehicles such as tea, instant noodles, fish sauce, yogurt and salt (Dary & Mora, 2002). Fortification of rice by coating (Rubin et al., 1977) or introducing the nutrients into artificial kernels (Ultra rice) (Lofti & Britton, 1997) resulted in unacceptable losses in the former and to high costs in the latter. Complementary foods or biscuits and beverages used in school feeding programs are other fortified products (Dary & Mora, 2002).

Zinc

Experience with the fortification of foods with zinc (Zn) is, at present, largely represented by the fortification of infant formulas, infant cereals and ready-to-eat breakfast cereals (IZINCG, 2004). Several Latin American countries have used or are currently using centrally processed complementary foods fortified with multiple micronutrients, including Zn and Mexico also has developed a fortified, milk-based, beverage mix targeted toward pregnant and lactating women and a national
voluntary fortification program for wheat and lime processed maize flours, although the efficacy has not been tested so far. (Rivera & Lutter, 2001; IZiNCG, 2004). Examples of candidate Zn fortification vehicles include rice, wheat and maize or condiments such as salt or fish sauce (IZiNCG, 2004).

Multiple micronutrients
A single micronutrient addition to an appropriate food vehicle is increasingly the less common approach in food fortification programs in developing countries due to concurrent micronutrient deficiencies. There are several food vehicles fortified with more than one micronutrient including wheat and maize flour as well as infant formulas fortified with Fe, B-vitamins and vitamin A in several countries (Layrisse et al., 1996; Lofti et al., 1996; Walter et al., 2001; Venkatesh Mannar & Sankar, 2004), rice usually fortified with B-vitamins and iron and sometimes with additional vitamin A in several countries (Lofti et al., 1996), fish sauce fortified with iodine and Fe in Thailand (Chavasit et al., 2003), sugar fortified with vitamin A and Fe in Guatemala (Viteri et al., 1995), monosodium glutamate with Fe and vitamin A in the Philippines (Lofti et al., 1996), instant noodles with Fe, iodine and vitamin A in Thailand (Chavasit & Tontisirin, 1998) and sprinkles containing zinc, folic acid, vitamin A and C, and iron to be added to weaning food and tested in several countries (MI, 2004).

Iron fortification
Fortification of food with Fe has been suggested as an effective method for the prevention of anemia (WHO, 1972) and the first food vehicles fortified with Fe were cereals, fish sauce and sugar (Elwood et al., 1968; Garby & Areekul, 1974; Viteri et al., 1978). More recently, the number of used vehicles for Fe fortification has increased, including cereal flours, infant weaning foods, salt, sugar, rice, curry powder, fish and soy sauce, bakery products, beverages, chocolate milk, margarine and water (Lofti et al., 1996). However, there are technical hurdles in fortifying the diet with Fe such as the selection of the fortificant Fe source which should be bioavailable and not cause sensory changes (Hurrell, 2002b).
Iron compounds

A variety of Fe compounds are available on the market but the main problem in choosing an Fe compound is that the water-soluble Fe compounds, which are the most bioavailable, often lead to the development of unacceptable color and flavor changes in the fortified food (Hurrell, 2002b). The large number of different potential Fe compounds for food fortification and their properties are listed in Table 1. Generally, the potential of adverse organoleptic changes in the fortified food is expected to be high when freely water soluble Fe compounds are used, low in the case of poorly water soluble compounds and negligible when water insoluble compounds are used (Hurrell, 2002b). Besides this difference between the groups of Fe compounds, there is also a large difference in their relative bioavailability (RBV) which depends largely on their solubility in the gastric juice (Hurrell, 2002b). Water soluble compounds, such as ferrous sulfate, dissolve instantaneously and have the highest RBV. Poorly water soluble compounds, such as ferrous fumarate, may have a high RBV as well due to complete but slower dissolution in the dilute acid of gastric juice. Water insoluble compounds are only poorly soluble in dilute acid and therefore never dissolve completely resulting in low and variable RBV. Based on this findings the first choice of a Fe compound would be a soluble compound; a good alternative would be a compound such as ferrous fumarate and the last choice would be an elemental or phosphate Fe source (Hurrell, 2002b). Alternatively, encapsulation of the more soluble compounds could help overcoming unwanted sensory changes. However, the RBV is also influenced by the acidity of the gastric contents, the presence of compounds in the meal facilitating the rate of dissolution of the fortification iron (such as different acids), chelating compounds, and the time the food remains in the stomach (Hallberg, 1985). In addition, iron absorption enhancers and inhibitors have been shown to influence not only native food Fe but also fortification Fe demonstrating another technical barrier to cope with (Hurrell, 2002b).
Table 1: Characteristics of some common Fe fortification compounds (Hurrell, 1997c; Dary, 2002; Hurrell, 2002b)

<table>
<thead>
<tr>
<th>Fe compound</th>
<th>Fe (%</th>
<th>Rat</th>
<th>Human</th>
<th>Relative cost¹</th>
<th>Potential food vehicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freely water soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrous sulfate x 7H2O</td>
<td>20</td>
<td>100</td>
<td>100</td>
<td>1.0</td>
<td>wheat flour (short storage), infant formula &amp; cereals, bread, pasta, maize flour, salt, sugar, milk, powder, fish sauce</td>
</tr>
<tr>
<td>Dried ferrous sulfate</td>
<td>33</td>
<td>100</td>
<td>100</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Ferrous gluconate</td>
<td>12</td>
<td>97</td>
<td>89</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Ferrous lactate</td>
<td>19</td>
<td>-</td>
<td>106</td>
<td>4.1</td>
<td>fish sauce</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>18</td>
<td>107</td>
<td>-</td>
<td>2.1</td>
<td>fish sauce</td>
</tr>
<tr>
<td>Ferrous ammonium sulfate</td>
<td>14</td>
<td>99</td>
<td>-</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Poorly water soluble/soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in dilute acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>33</td>
<td>95</td>
<td>100</td>
<td>1.3</td>
<td>infant formula &amp; cereals, processed cereals, maize flour, coffee, chocolate drink powder, infant cereals</td>
</tr>
<tr>
<td>Ferrous succinate</td>
<td>35</td>
<td>119</td>
<td>92</td>
<td>4.1</td>
<td>infant cereals</td>
</tr>
<tr>
<td>Ferric saccharate</td>
<td>10</td>
<td>92</td>
<td>74</td>
<td>5.2</td>
<td>infant cereals</td>
</tr>
<tr>
<td>Ferric glycerophosphate</td>
<td>15</td>
<td>93</td>
<td>-</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Ferrous citrate</td>
<td>24</td>
<td>76</td>
<td>74</td>
<td>3.9</td>
<td>wheat flour, sugar, eggs</td>
</tr>
<tr>
<td>Ferrous tartrate</td>
<td>22</td>
<td>77</td>
<td>62</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Water insoluble/poorly soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in dilute acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>25</td>
<td>45-58</td>
<td>21-74</td>
<td>2.3</td>
<td>infant cereals, chocolate drink powder, processed cereals, infant cereals, wheat flour, salt, sugar, chocolate drink</td>
</tr>
<tr>
<td>Ferric orthophosphate</td>
<td>28</td>
<td>6-46</td>
<td>25-32</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Elemental Fe powders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrolytic</td>
<td>97</td>
<td>16-70</td>
<td>75</td>
<td>²</td>
<td>wheat flour, infant cereals, maize meal, processed cereals, candy</td>
</tr>
<tr>
<td>H-reduced</td>
<td>97</td>
<td>13-54</td>
<td>13-148</td>
<td>²</td>
<td></td>
</tr>
<tr>
<td>CO-reduced</td>
<td>97</td>
<td>12-32</td>
<td>ND</td>
<td>²</td>
<td></td>
</tr>
<tr>
<td>Atomized</td>
<td>97</td>
<td>ND</td>
<td>ND</td>
<td>²</td>
<td></td>
</tr>
<tr>
<td>Carbonyl</td>
<td>99</td>
<td>35-66</td>
<td>5-20</td>
<td>²</td>
<td></td>
</tr>
<tr>
<td>Protected compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaFeEDTA</td>
<td>14</td>
<td>-</td>
<td>28-416</td>
<td>6</td>
<td>sugar, curry powder, fish &amp; soy sauce, maize flour</td>
</tr>
<tr>
<td>Ferrous bisglycinate</td>
<td></td>
<td></td>
<td>100-400</td>
<td>10</td>
<td>milk, dairy products</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.34</td>
<td>-</td>
<td>100-700</td>
<td>-</td>
<td>rice flour, cookies</td>
</tr>
</tbody>
</table>

¹ Relative to ferrous sulfate x 7H2O = 1.0, for the same level of total Fe
² In general, less expensive than ferrous sulfate. Cost of different powder types varies about sevenfold, with carbonyl Fe being the most expensive. ND, not determined.
Estimation of iron bioavailability

Iron bioavailability is the result of many preceding steps which can be divided in three parts (Wienk et al., 1999). First, the digestibility and more specific, the solubility of Fe in digesta is a determinant for its subsequent bioavailability. A second determinant is Fe absorption and its delivery to the circulation and a third determinant is the processing of Fe once it has entered the circulation and its incorporation into a functional entity. Fe bioavailability is always assessed during one or more of these three steps and there is a continuing need for reliable methods to assess the bioavailability of Fe-fortification compounds (Forbes et al., 1989).

Iron solubility

Fe solubility can be measured in vitro and usually involves a simulated gastric digestion followed by a measurement of soluble or dialyzable Fe (Miller et al., 1981). These relatively simple, rapid, inexpensive methods are alternatives to human absorption studies and are often useful to predict the direction of the absorptive response in man but not the magnitude (Hurrell et al., 1988; Hurrell et al., 1989b). The most important limitation of every Fe solubility study that is used to predict Fe bioavailability is its inability to pronounce upon subsequent absorption and incorporation of Fe and that no methods are available to study heme Fe bioavailability by in vitro solubility (Wienk et al., 1999).

Iron absorption

Several methods can be used for the determination of Fe absorption including methods that use native Fe, the application of radioisotopes of Fe (\(^{55}\)Fe and \(^{56}\)Fe) and methods using stable Fe isotopes (\(^{54}\)Fe, \(^{57}\)Fe and \(^{58}\)Fe) (Wienk et al., 1999).

Chemical balance studies using native Fe measure the amount of Fe that is absorbed or retained by the body, the so called apparent absorption which is calculated by subtraction of fecal and urinary Fe from ingested Fe (Wienk et al., 1999). These measurements are easy to do in small animals housed in metabolic cages and are used in humans mainly because many nutrients can be measured in the same samples, a reflection of Fe absorption from the whole diet during a prolonged period can be obtained, and the use of radioactivity can be avoided (Ivaturi & Kies, 1992; Rosado et al., 1992; Hunt et al., 1995). However, balance techniques are cumbersome and they are imprecise due to relatively low Fe absorption (Hurrell,
An alternative is measuring postabsorption plasma Fe which is commonly used to evaluate pharmacological doses of Fe (Wienk et al., 1999) and only a few human studies exist in which changes in plasma Fe were induced by dietary Fe or meal components given together with Fe (Pla & Fritz, 1972). However, food Fe usually produces only small changes in the plasma level (Hurrell, 1997b).

The major progress in understanding Fe bioavailability in man, resulted from the development of the extrinsic tag radioiron technique with $^{55}$Fe and $^{59}$Fe (Cook et al., 1972). Radioiron can be determined in feces and urine, in digesta of rats, in plasma or by whole-body counting in rats and humans measuring the retained radioactivity (Wienk et al., 1999).

Due to the concern regarding the use of radioisotopes in human subjects, especially children and pregnant women, stable isotopes have been used to determine fecal balance by neutron-activation analysis or by mass spectrometry (Janghorbani et al., 1980; Fairweather-Tait et al., 1989).

Another possibility to measure Fe absorption is by using the Caco-2 cell line which originates from humans and show a remarkable similarity to human studies (Alvarez-Hernandez et al., 1991).

Iron incorporation

The most widely used animal method, the hemoglobin-repletion bioassay, which is the recommended method according to the Association of Official Analytical Chemists (AOAC, 1997) to estimate Fe bioavailability, compares the hemoglobin (Hb) response in anemic rats given graded quantities of the fortification Fe source with that obtained from ferrous sulfate (Forbes et al., 1989). This method, slightly modified and using the slope ratio technique, has shown excellent agreement with clinical measurements (Forbes et al., 1989). However, another study using radioiron has found that the Fe absorption rate is normally much higher in rats so that enhancers and inhibitors which have a profound effect in man give only a limited response in rats (Reddy & Cook, 1991). Nevertheless, the rat Hb-repletion model has some clear advantages compared to human isotope studies: it is relatively simple to perform, large numbers of samples can be screened, responses to treatment are uniform and it is inexpensive (Wienk et al., 1999) but it gives results of non-heme iron absorption that can be extrapolated to humans only with caution (Reddy & Cook, 1991).
Studies with blood using radioisotopes are performed in animals and humans (Wienk et al., 1999). Using the double-isotope method Fe absorption in human is measured from two separate meals extrinsically labeled with either $^{55}$Fe or $^{59}$Fe and consumed on consecutive days (Cook et al., 1972). Two weeks after consuming the labeled meal, the radioactivity retained by the body is quantified either using a whole body counter or by measuring the radioactivity incorporated into circulating red cells and assuming this to be 80% of the total absorbed Fe in healthy subjects having a normal Fe status (Hosain et al., 1967). To compare Fe absorption between different subjects a reference dose of 3 mg Fe administered to all subjects can be used (Magnusson et al., 1981).

The double-isotope method can also be used with stable isotopes (Kastenmayer et al., 1994) being particularly useful in the assessment of vulnerable groups. However, this methodology using $^{57}$Fe and $^{58}$Fe is more expensive and requires mass spectrometry for quantification (Hurrell, 1997b). This method has successfully been applied in infants, young children and women to estimate the absorption of fortification Fe and the influence of Fe absorption enhancers and inhibitors (Davidsson et al., 1994; Davidsson et al., 2000; Fidler et al., 2003a; Fidler et al., 2003b; Fidler et al., 2004a; Fidler et al., 2004c; Walter et al., 2004).

For the study of fortification Fe, in vitro solubility methods can usefully predict the direction of a response but not the magnitude and Hb repletion studies in rats may be useful in predicting the relative bioavailability (Forbes et al., 1989; Hurrell, 1997b; Wienk et al., 1999). Ultimately, human subjects have to be studied in order to corroborate results from in vitro and/or animal studies.

**Organoleptic problems**

Many Fe compounds are colored and cannot be used to fortify light-colored foods, such as salt and rice, and the more soluble Fe compounds often react with substances in foods, causing discoloration (Hurrell, 1997c). Infant cereals fortified with ferrous sulfate have been shown to turn gray or green and dark blue in the presence of bananas (Hurrell, 1984) and addition of ferrous sulfate, ferrous lactate, ferrous gluconate, and ferric ammonium citrate, as well as the less soluble ferrous fumarate and ferric citrate, produced off-colors when added to a chocolate milk drink probably due to the implication of phenolic compounds (Douglas et al., 1981). Off-
flavor is another problem when soluble compounds are used. It can result from the metallic taste of the Fe itself, particularly in beverages, or from fat oxidation promoted by soluble Fe compounds in cereals or milk products during storage (Demott, 1971; Edmondso et al., 1971; Hurrell, 1997c). In contrast, fortification of whole wheat flour with the water insoluble compounds, ferric pyrophosphate and reduced elemental Fe, resulted in acceptable organoleptic properties after 7 weeks of storage (Hurrell, 1984). In addition to cause unacceptable changes in color and flavor Fe compounds may also provoke precipitation, such as when added to fish sauce (Garby, 1985) or when Fe fortified sugar is added to tea (Disler et al., 1975).

Therefore, ferrous sulfate is only commonly used in infant formula, bread, pasta and wheat flour stored for less than 2 months (Hurrell, 2002b). Other soluble compounds provoke similar sensory changes and are more expensive (Hurrell, 2002a). Therefore, foods such as infant cereals, chocolate drink powders, rice, salt, wheat flour and breakfast cereals are most commonly fortified with elemental Fe, ferric pyrophosphate and ferric orthophosphate which provoke less sensory changes but which has for long been considered not to be favorable since these compounds are poorly soluble in dilute acid and therefore have a low and variable relative bioavailability in man (Hurrell, 1998). However, it has recently been shown that these compounds can be acceptable if added at higher levels to overcome their lower relative bioavailability (Zimmermann et al., 2004b). Another option, if they are organoleptically acceptable, is to use compounds such as ferrous fumarate and ferric saccharate which, although insoluble in water, are soluble in dilute acid (Hurrell, 1998). These compounds are used to fortify infant cereals (Hurrell et al., 1989a), chocolate drink powder (Hurrell et al., 1991) and wheat and corn flour (Garcia-Casal & Layrisse, 2002). Chelate compounds such as NaFeEDTA and ferrous bisglycinate have become more important as fortificants. In addition to high bioavailability, NaFeEDTA does not promote fat oxidation in stored wheat flour, but may cause unacceptable color changes in some food vehicles (Hurrell, 1997c). This compound does not precipitate peptides during storage of fish sauce and soy sauce and the taste and color is satisfactory, but can be degraded by ultraviolet rays from sunlight in liquid products (Fidler et al., 2004b). In contrast, ferrous bisglycinate promotes fat oxidation in cereal foods (Bovell-Benjamin et al., 1999) and causes undesirable color reactions in some foods but is suitable for the fortification of liquid milk and other dairy products (Hurrell et al., 2004). Hemoglobin, although highly bioavailable, is an
unattractive Fe compound for many applications due to the intense red color (Hurrell, 2002a).

**Application and experience**

*Cereal products*

Cereal flour is the most common food vehicle for Fe fortification but others, such as noodles, rice, and various sauces, have also been used (Baltussen et al., 2004). The addition of micronutrients to wheat flour including Fe was first introduced in the United States and some European countries in the 1940s, followed by Chile in the early 1950s (Hertrampf, 2002). During the 1960s, legislation regarding the addition of Fe and B vitamins similar to U.S. levels was proposed in a number of Latin American countries and since the 1990s all countries in the region, except Argentina and Uruguay, introduced mandatory wheat flour fortification and Venezuela started a national fortification program using precooked maize flour (Hertrampf, 2002). Active efforts are under way to overcome the technical limitations of fortifying nixtamalized maize flours commonly consumed in Mexico and Central America (Hertrampf, 2002). In the Middle East and North Africa, several countries agreed that the fortification of wheat flour with Fe should be introduced and many countries have implemented this fortification program (Alnwick, 1998).

Although elemental Fe powders generally have a lower bioavailability than ferrous sulfate, they have been used for flour fortification for > 50 y and continue to be the most widely used Fe compounds due to their advantage of causing few, if any, color and flavor problems in stored food vehicles and because it is inexpensive and suitable for wheat and maize flour fortification (Hurrell et al., 2002). Elemental iron powders including carbonyl iron, electrolytic iron and reduced iron, are manufactured through several different methods of production resulting in products with distinct and different physical properties (SUSTAIN, 2001). According to the SUSTAIN Guidelines for Iron Fortification of Cereal Food Staples (SUSTAIN, 2001), electrolytic iron is the best choice of the elemental iron powders with a relative bioavailability approximately half of that of ferrous sulfate. However, a recent rat study comparing the bioavailability of 6 elemental iron powders showed the highest RBV for carbonyl iron with 64% that of ferrous sulfate, followed by two types of electrolytic iron with an RBV of 46 and 54%, respectively (Swain et al., 2003). H-reduced, reduced and CO-reduced iron powders had RBVs of 42, 24 and 21%. The significantly higher RBV of
carbonyl iron in this study might be explained by the smaller particle size and the spherical particle morphology compared to the other compounds. Human studies with carbonyl iron added to bread indicated a RBV of only 22% (Hallberg et al., 1986), although a RBV of 75% has been reported in supplementation studies (Devasthali et al., 1991). A recent human study comparing the efficacy of H-reduced and electrolytic iron to ferrous sulfate when baked into cookies found a relative bioavailability of 49% and 79%, respectively, suggesting higher bioavailability of electrolytic iron than expected most probably due to the low phytic acid content of the cookies (Zimmermann et al., 2005). According to current recommendations, electrolytic iron should be added to wheat flour at double the level of ferrous sulfate and atomized and CO-reduced powders are not recommended for wheat flour fortification (Hurrell & Lynch, 2004).

Although most countries use elemental Fe as fortificant for wheat flour, some countries use ferrous sulfate or ferrous fumarate (Hertrampf, 2002). Due to its high bioavailability and low cost dried ferrous sulfate has been recommended for bakery flour, semolina and other types of low extraction wheat flours, which are normally used within one to two months after production (SUSTAIN, 2001). It is not appropriate to use this compound in products stored for extended periods due to its promotion of oxidative rancidity of native or added fats, and its potential to change color and flavor over time (SUSTAIN, 2001). Because ferrous fumarate has a bioavailability similar to that of ferrous sulfate and it is insoluble in water and therefore causes fewer organoleptic problems, this compound is another good choice (SUSTAIN, 2001). However, it is more costly than ferrous sulfate (SUSTAIN, 2001). The impact of Fe fortification of wheat and maize flour fortified with ferrous fumarate has been measured only in Venezuela demonstrating a significant reduction in anemia and ID in children after two years of implementation but anemia prevalence increased again to the level before the fortification program started 5 years after implementation probably due to a deterioration of living conditions, an increase in wheat flour and a decrease in maize flour (additionally fortified with vitamin A) consumption and a partial substitution of a high bioavailable (ferrous fumarate) for a less available form of Fe (elemental Fe) (Layrisse et al., 1996; Garcia-Casal & Layrisse, 2002).

Another Fe compound used to fortify whole-wheat flour (atta) and high extraction corn flour is NaFeEDTA (INACG, 2002). This Fe chelate has the advantages that Fe
absorption is not as sensitive to inhibiting substances in food as from ferrous sulfate, that it is highly bioavailable due to its solubility in water and that it has a high stability constant and does not provoke fat oxidation in wheat flour (MacPhail et al., 1985; Bothwell & MacPhail, 2004). Inhibitory staple foods such as corn masa flour showed increased Fe bioavailability when fortified with NaFeEDTA compared to fortification with ferrous sulfate (Davidsson et al., 2002). Maize meal fortified with NaFeEDTA is being successfully marketed in Kenya (Bothwell & MacPhail, 2004). A recent in vitro trial with NaFeEDTA fortified whole-grain wheat chapatis showed a significant increase in dialyzable Fe and absorbed Fe by Caco-2 cells compared to chapatis made with ferrous sulfate (Kloots et al., 2004). However, human absorption or efficacy studies are needed to prove these results. An absorption study, feeding NaFeEDTA-fortified and ferrous sulfate fortified rice to Fe deficient subjects in the Philippines, has shown that both Fe compounds are effective iron fortificants for rice, but authors don’t give any information on the organoleptic quality of the rice (Trinidad et al., 2002).

Beside the chelate compound NaFeEDTA, two types of amino acid chelates, ferrous bisglycinate (Ferrochel®) and ferric trisglycinate ("taste-free" iron chelate), are available for food fortification (Dary et al., 2002). Although bioavailability of ferrous bisglycinate is high and partially prevents the inhibitory effect of phytates (Layrisse et al., 2000) it is expensive and has a tendency to cause unwanted color reactions and fat oxidation in stored cereal fours (Torres et al., 1996; Dary et al., 2002). In contrast, ferric trisglycinate causes fewer reactions in foods, but its bioavailability is much lower than that of ferrous bisglycinate (Bovell-Benjamin et al., 2000).

**Dairy products**

Milk-based items fortified with premixes containing several micronutrients including ferrous sulfate targeting foods to groups with high risk of ID such as infants, children and pregnant and lactating women are applied in Mexico, Peru and Chile (Lopez de Romana, 2000; Rivera et al., 2000; Kain & Uauy, 2001). The consumption of the fortified milk seemed to be able to prevent IDA in Chilean infants (Torrejon et al., 2004). Ferrous bisglycinate has been shown to have potential for milk fortification as its absorption was higher than from milk fortified with ferrous sulfate in an absorption study (Olivares et al., 1997). The efficacy of ferrous bisglycinate was proven in a study conducted in Brazilian school children consuming a whey drink fortified with
ferrous bisglycinate and other micronutrients resulting in a significant reduction of the anemia prevalence after 6 months (Miglioranza et al., 2003).

Condiments
NaFeEDTA has successfully been used to fortify fish sauce (Fidler et al., 2003a; Thuy et al., 2003) and soy sauce (Mannar & Gallego, 2002; Fidler et al., 2003a) in Asia, curry powder in South Africa (Ballot et al., 1989) and sugar in Guatemala (Viteri et al., 1995). However, sugar fortification was difficult in real-life situations due to a too high price increment, segregation of the NaFeEDTA and the risk of organoleptic changes when sugar is used as an ingredient by different industries (Dary, 2002). Monosodium glutamate has been fortified with ferrous sulfate encapsulated in zinc stearate and with ferric phosphate and showed good distribution of the Fe and good stability of the vitamin A added as well after one year of storage (Bauernfeind & Timreck, 1978). Further testing is required for the efficacy of these two systems when consumed with meals (Zoller et al., 1980).

Other products
Some Latin American countries add Fe as ferrous sulfate or ferrous fumarate to blended complementary foods for young children and foods used for school lunches without any problem of organoleptic changes (Dary et al., 2002). Data collected from the processed complementary food program in Peru suggest a reduction in the prevalence of anemia (Lopez de Romana, 2000). In another intervention, the effect of bovine hemoglobin-fortified cookies as part of the normal school meal program was evaluated in Fe deficient preschool children in Brazil and showed a significant increase in Hb after three months with no measurable side effects or taste alterations reported (Nogueira et al., 1992). However, important considerations and guarantees should be given as to the safety and sterility of heme Fe due to its production from livestock (Beinner & Lamounier, 2003). Similarly, candies fortified with elemental Fe given to children at school in Indonesia showed good acceptability and a decrease in the prevalence of anemia (Sari et al., 2001). In earlier years, coffee has been fortified with ferrous fumarate without causing organoleptic changes (Johnson & Evans, 1977). However, its absorption was only tested in animal studies.
Iron fortification of salt has been tried since many years and has been shown to have many advantages but to be very challenging at the same time.

**Salt as fortification vehicle**

As discussed above, to be successful, fortification programs must include fortified foods which are consumed in adequate amounts by all those in the population who need the additional micronutrients and this is often not the case, because poorer and undernourished segments of the population are more likely to produce their own food and purchase fewer fortified products (Allen, 2003). In most African countries, for example, staple cereals are not centrally processed and fortification of staple food would have to take place at small-scale mills complicating this process (MI, 2004) and not being appropriate in subsistence settings (Gibson, 2004).

Already in 1972, Levinson (1972) suggested considering unconventional vehicles such as salt, sugar, seasonings and cooking oil, due to no access to other centrally processed foods, low purchasing power and sensitivity to higher-priced fortified food of the poorer population. A study in Indonesia has shown that salt and monosodium glutamate (MSG) would be the preferred vehicles for micronutrient fortification because of a high consumption frequency and the absence of a relationship with socio-economic status in urban and rural areas (Melse-Boonstra et al., 2000). Studies from Côte d'Ivoire suggest that only fortification of salt or bouillon cubes would be feasible for reaching the entire population since staple foods differ between the different food zones and are not centrally produced (Staubli-Asobayire, 2000). It is estimated that salt is the most consumed processed food by the poorest segment of the population followed by sugar and vegetable oil (Alnwick, 1998). Salt is one of the few commodities consumed at roughly the same level throughout the year by all members of a community which makes it possible to determine accurately the appropriate level at which a particular nutrient is to be added and to control its intake (Venkatesh Mannar & Diosady, 1998). In addition, salt is one of the few food vehicles which is often centrally processed in poor countries with the distribution regulated to some degree by the government (Alnwick, 1998; Venkatesh Mannar & Diosady, 1998) and salt is already a well-known fortification agent as it is used worldwide as a vehicle for iodine (Melse-Boonstra et al., 2000).

There are also some substantial technical barriers to fortification including adverse effects on the sensory qualities of foods, nutrient-nutrient interactions and poor
bioavailability of some fortificants (Allen, 2003). Salt tends to be extremely difficult to fortify due to its white color and the often poor quality in developing countries. The quality of salt can be divided into 3 categories (Lofti et al., 1996): 1) salt that is already refined and well packed to match the requirements for fortification; 2) salt that is partially refined but does not meet specifications for fortification and 3) salt that is not refined. According to The Micronutrient Initiative (Lofti et al., 1996) salt used for iron fortification will require a minimum purity of 99% (dry basis), a maximum moisture content of 0.5%, a maximum magnesium level of 0.05% and the salt should be fine grained and of uniform size (0.5-1mm). However, salt from developing countries most often contain less than 99% NaCl going down as far as to 91%, as analysis of Moroccan and Ivorian salt have shown with the most common impurities of calcium- and magnesium salts (Narasinga Rao, 1994; Ranganathan et al., 1996), (R. Wegmüller, unpublished data, 2001-2004). Those salts often consist of rather large particles and show a broad particle size distribution (R. Wegmüller, unpublished data, 2001 -2004). In addition, salt in tropical countries, like Côte d'Ivoire, usually show a high moisture content of up to 4% (R. Wegmüller, unpublished data, 2001-2004) and the presence of a high level of magnesium chloride in the salt increases its hygroscopicity and can accelerate chemical reactions (Lofti et al., 1996). Similar results of poor salt quality were found in India, where magnesium, insoluble solid and moisture content were high in most of the salts (Ranganathan et al., 1996; Sivakumar et al., 2001).

Dual fortification of salt

As mentioned in the above section, in some countries salt is likely to be the only food vehicle that is purchased even in remote rural areas and is consumed regularly in constant amounts throughout the year by the whole population, particularly in areas of subsistence farming, e.g. in rural Africa. Iodization of salt is implemented in most countries today and the addition of Fe during salt iodization would not only be beneficial to combat and prevent Fe deficiency but would at the same time increase iodine utilization due to interactions in the metabolic processes of this two micronutrients (Hess et al., 2002a; Hess et al., 2002b; Hess & Zimmerman, 2004). It is estimated that the diet of more than a billion people in developing countries is deficient in the two nutrients iodine and Fe, and dual fortified salt (DFS) could have a
significant public health impact in preventing these deficiencies (Diosady & Venkatesh Mannar, 2000).

Challenges and experience with single fortification of salt with iron

Almost all the development work on fortification of common salt with Fe has been done in India over the past 30 years. Finding a Fe source that is suitable to fortify salt is challenging, due to its white color and the poor quality of salt often encountered in developing countries. The criteria for such a compound are: stability when mixed with salt and no color development, satisfactory bioavailability when salt is added to food and processed, no change of color or taste in food to which it is added and stability during storage and transportation (Narasinga Rao, 1985).

Addition of ferrous sulfate or other soluble Fe compounds, such as ferrous and ferric ammonium sulfate, ferric ammonium citrate and ferric EDTA to crude salt resulted in a brown or yellow color change either immediately or within a few days of mixing as a result of oxidation and hydrolysis of the Fe compound (Narasinga Rao, 1985). Due to these results, some of the insoluble sources, including phosphates and reduced Fe, were investigated because they were expected to be stable when added to crude salt with a high moisture content (Narasinga Rao, 1985). Phosphate compounds are white and when mixed to salt were found to be highly stable for several months under a variety of storage conditions but absorption of the tested compounds (sodium iron pyrophosphate, ferric orthophosphate, ferric pyrophosphate) was judged unacceptably low, particularly when salt was consumed as part of a cereal-based meal (Narasinga Rao et al., 1972). Another insoluble and white compound is ferrous citrate, from which Fe was shown to be as available as from ferrous sulfate in a absorption study when given alone or when added to a rice-based meal, but it developed a greenish yellow color when added to salt (Narasinga Rao et al., 1978). Reduced Fe powder produced a brown color readily when mixed with crude salt (Narasinga Rao, 1985).

Due to these results two alternative approaches were investigated: to identify a chemical agent that would prevent discoloration when ferrous sulfate, ferrous ammonium sulfate, or ferrous citrate was used or to identify a chemical agent that would enhance Fe absorption from a stable insoluble Fe compound like ferric phosphate (Narasinga Rao & Vijayasarathy, 1975). Only two compounds were found to prevent color development, orthophosphoric acid (OPA) and sodium
hexametaphosphate (SHMP), and prevention was only effective when these coordinating agents were present in excess (2 mol/mol FeSO₄). However, ferrous sulfate was oxidized when added to salt together with SHMP or OPA resulting in the reaction of ferric Fe with phosphate yielding insoluble ferric phosphate and thereby decreasing the bioavailability. In the second approach a number of absorption promoters were tested and ascorbic acid was found to develop a pink color as had been shown before (Sayers et al., 1974). Only sodium acid sulfate (NaHSO₄) increased absorption from FePO₄ significantly to \( \approx 80\% \) of that of ferrous sulfate. In addition, this salt fortified with FePO₄ and NaHSO₄ in a 1:2 molar ratio was found to be stable up to 8 months and Fe absorption did not deteriorate during storage. In addition, this salt was found to be acceptable and it did not alter color or taste in prepared dishes. An efficacy study in school children has shown a significant increase in Hb concentration after one year in the group getting the fortified salt (Nadiger et al., 1980) and a field trial among a rural population showed the same effect (Working Group Report, 1982). This was the first formula satisfying bioavailability and color stability. This formulation was improved by using the less expensive FeSO₄ instead of FePO₄ with a mixture of OPA along with NaHSO₄ (Narasinga Rao & Vijayasarathy, 1978) and has shown the same efficacy as the original formula in a field trial (Working Group Report, 1982). However, several problems were encountered during the large scale manufacturing including yellow discoloration within a few days and corrosion of the fortification plant due to the use of the corrosive OPA and the strongly acidic NaHSO₄ (Ranganathan, 1992). Therefore, an attempt to develop an alternative formula using ferrous sulfate and polyphosphates which have a high iron chelating ability was undertaken (Ranganathan, 1992). It was found that salt fortified with ferrous sulfate heptahydrate and SHMP did not develop any color throughout one year of storage, color and taste of prepared food were not altered, and percentage absorption was higher than from ferrous sulfate.

Even though technology for Fe fortification of salt was developed and field trials confirming the effectiveness of the formulation were completed in the 1970s, the program did not find large-scale application partly because it was overtaken by a major thrust in several developing countries, including India, to iodate salt to control IDD (Venkatesh Mannar & Diosady, 1998). After the implementation of iodization
programs the first attempts to incorporate iodine and Fe in the same salt were made in the mid 1980s in India (Venkatesh Mannar & Diosady, 1998).

**Challenges and experience with dual fortified salt**

Dual fortification of salt with Fe and iodine for simultaneous prevention of IDA and IDD has been a goal of researchers for at least 20 years. The major problem is the interaction between iodine and Fe which results in a loss of iodine (Diosady & Venkatesh Mannar, 2000). Potassium iodate is the iodine compound most often used in salt iodization programs in developing countries but unfortunately most of the added iodine can be lost from impure iodated salt during extended storage at high temperature and humidity (Diosady et al., 1997; Diosady et al., 1998), which are conditions often encountered in developing countries. If Fe is added to the salt in the most bioavailable ferrous form (Fe\(^{2+}\)) it can be converted to the ferric form (Fe\(^{3+}\)) by oxidation resulting in lowered bioavailability, poor taste and unacceptable discoloration (Venkatesh Mannar, 1987). This reaction is accelerated by alkaline conditions, oxidizing agents, including air, salt impurities, such as magnesium chloride and magnesium sulfate, and high humidity, exactly being the prevailing conditions in many developing countries (Venkatesh Mannar, 1987). If salt is dual fortified with ferrous Fe and iodate, ferrous compounds can be oxidized by iodate resulting in the loss of iodine due to evaporation:

\[2I^- + 10Fe^{2+} \rightarrow I_2 + 10Fe^{3+}\]

To date, 6 different approaches have been investigated in order to achieve a stable dual fortified salt with a satisfying bioavailability.

1. **Ferrous sulfate and SHMP**

Ferrous sulfate was found to be protected from oxidation by SHMP with NaHSO\(_4\), and OPA in Fe fortified salt as discussed in the section above (Narasinga Rao & Vijayasarathy, 1975). The same authors have also extensively investigated dual fortification of salt looking at the effects of CaCO\(_3\) and several polyphosphates (SHMP, sodium tripolyphosphate, tetrasodium pyrophosphate) on the stability of salt fortified with ferrous sulfate and either potassium iodide (KI), potassium iodate (KIO\(_3\)),
or calcium iodate (Ca(IO₃)₂) (Narasinga Rao, 1994). Without the addition of a stabilizer iodine in the DFS decreased rapidly in all salts fortified with the three different iodine compounds and the addition of CaCO₃ did not improve its stability. All three tested polyphosphates stabilized the iodine in salt fortified with FeSO₄ and KI to some degree, but two of them led to a brown discoloration during storage and only SHMP developed no color change. The optimum level for SHMP was found to be 1% when added to salt fortified with 1 mg Fe and 40 µg iodine per g salt and iodine was stable for 6 months and losses comparable to iodized salt beyond 6 months. The availability of iodine from this salt was comparable to iodized salt and was not affected by SHMP or Fe. Fe absorption was slightly higher but not significantly different from the DFS compared to FeSO₄ when consumed with a rice-based meal and acceptability testing showed no changes in color or taste of the food. The addition of SHMP presumably chelates all the Fe in this formulation and keeps the iron soluble, thus preventing its interaction with the iodide. The intake of SHMP contributes to only 4% of the acceptable daily intake of phosphorus and therefore this salt can be judged as safe for human consumption. The safety and long-term feeding in relation to calcium and phosphorus metabolism was tested in rats and was found that SHMP had no effect on calcium and phosphorus metabolism, except for an increase in serum phosphorus (Madhavan Nair et al., 1998b), which was not confirmed by later data in residential school children showing no difference in the serum phosphorus levels between the DFS and IS group (Madhavan Nair et al., 1998a).

The large scale production of dual fortified salt containing ferrous sulfate heptahydrate, SHMP and KI or KIO₃ showed that the spray mixing process was unsuitable due to discoloration and rapid iodine loss (Ranganathan et al., 1996). No such problems were encountered with the dry mixing process and dual fortified salt retained the original color and iodine content even during prolonged storage and transportation (Ranganathan et al., 1996). However, experience has shown that it is difficult to ensure iodine stability in salt fortified with Fe and iodine if the moisture content is > 2% and the magnesium content exceeds 0.1% (Venkatesh Mannar et al., 1989b; Ranganathan et al., 1996).

Field testing failed to show an impact of this DFS (Sivakumar et al., 2001), owing to confounding factors such as malaria and the consumption of tamarind which had been shown to reduce Fe bioavailability (Narasinga Rao & Prabhavathi, 1982).
following study in residential school children over two years was not able to show an overall improvement of Hb, but the authors concluded that DFS improved the Hb levels of anemic children (Sivakumar et al., 2001).

2. Formulation of an Indian Company
An Indian Company, Sunders Chemicals, developed a stable dual fortified salt containing 1000 ppm Fe and 30 ppm iodine and a stabilizer (Rajagopalan & Vinodkumar, 2000). Although the exact constituents are not reported, the company claims that the product is stable for more than one year and an efficacy trial conducted in tea pickers in southern India showed significant improvement in hemoglobin levels and work output compared to the control group (Rajagopalan & Vinodkumar, 2000).

3. Ferrous fumarate
Venkathesh Mannar et al. (1989a) showed that dual fortification of purified salt with ferrous fumarate and KI without the use of a stabilizer is feasible and does not deteriorate with time provided it is kept sealed in water-proof packing.

4. Encapsulated potassium iodide and/or encapsulated ferrous fumarate
Diosady et al. (2002) tested salt dual fortified with KI or KIO₃ and with ferrous sulfate or ferrous fumarate and the effect of stabilizer on the stability of iodine and Fe when stored under different conditions. They have shown that none of the combinations of iron and iodine compounds was stable at elevated temperatures (40°C) resulting in a loss of all the iodine after 6 months of storage. SHMP slowed down the iodine loss, whereas magnesium chloride greatly accelerated this process. Salt fortified with ferrous fumarate and KI was reasonably stable but only if the salt was highly purified and if it was stored for periods not exceeding three months.

Based on these results the University of Toronto, under the sponsorship of the Micronutrient Initiative (MI) and the International Development Research Centre (IDRC) used dextrin microencapsulation to create a barrier between ferrous fumarate and KI (Diosady & Venkatesh Mannar, 2000). Through spray drying of the iodine compound spherical particles were produced, each of which containing 0.5-2% KI, evenly distributed throughout the particle. While the iodine remaining on the surface of the particle remains vulnerable to oxidation, and iodine loss, this represents a very
small fraction of the total iodine present. Dual fortified salt prepared by mixing dextrin encapsulated KI and ferrous fumarate (1 mg Fe/g salt) using refined salt was stable for 12 months even at 40°C and 100% relative humidity, although some darkening occurred due to oxidation of the fumarate. An absorption study has shown that Fe is well absorbed from DFS but influenced by the composition of the meal and that urinary iodine excretion is maintained in the normal range (Sattarzadeh & Zlotkin, 1999). Field tests in Ghana have demonstrated the consumer acceptance and the effectiveness of this approach for the simultaneous elimination of iodine deficiency and maintenance or improvement of the hematological status of pregnant women and children, respectively (Diosady & Venkatesh Mannar, 2000).

The development of this salt has continued over the last years and a new formulation has recently been tested under storage and field conditions in coastal and highland regions of Kenya (Oshinowo et al., 2004). This dual fortified salt containing ferrous fumarate (1 mg Fe/g salt) microencapsulated with a combination of binders and coloring agents and coated with soy stearine and either nonencapsulated KIO₃ or KI microencapsulated with dextrin and coated with soy stearine packed in sealed polyethylene bags showed acceptable iodine retention during the three months study in most of the mixtures. Recent bioavailability tests conducted in animals have shown that the iron in DFS containing encapsulated ferrous fumarate is well absorbed, and that encapsulation does not reduce its bioavailability (MI, 2004). The formulation is currently tested in two large-scale trials in Nigeria and Kenya and sensory acceptability tests were successful and plans for market entry and testing are underway (MI, 2004). MI plans to take the DFS to scale in about 10 countries over the next five years providing a third of daily Fe requirements (MI, 2004).

5. Encapsulated ferrous sulfate

Another approach was investigated by Zimmermann et al. (2003b) which showed that salt dual fortified with ferrous sulfate (1mg Fe/g salt) encapsulated with partially hydrogenated vegetable oil and KI was stable during the dry season but developed a yellow discoloration during the damp season in northern Morocco. Iodine content was stable during both seasons and this salt has shown to be highly efficacious in reducing IDA.
6. Micronized ferric pyrophosphate

Another formulation of DFS using the less bioavailable ferric pyrophosphate compound but adding double the amount of iron (2 mg Fe/g salt) and using a micronized form (mean particle size \( \approx 2.5 \mu m \)) has recently been tested in northern Morocco (Zimmermann et al., 2004b). Results showed that salt fortified with micronized ferric pyrophosphate and KIO3 was stable during a storage period of 10 months and reduced the prevalence of IDA from 30% at baseline to 5% after 10 months in the group receiving the DFS.

Salt appears to be one of very few feasible food vehicles for iron fortification in poor countries with subsistence farming. Salt is widely used as vehicle for iodine fortification so that for the simultaneous fortification with iron many problems need to be overcome in order to develop a stable and bioavailable dual fortified salt. The above mentioned attempts of producing dual fortified salt demonstrate that the major problems with dual fortified salt, including discoloration, iodine loss and bioavailability of the Fe, may be overcome by encapsulation of a soluble bioavailable Fe compound or by using an insoluble Fe compound at a higher fortification level and/or with a small particle size which may increase bioavailability.
MICROENCAPSULATION OF MICRONUTRIENTS IN FOOD FORTIFICATION

Rational and objective

The science of encapsulation deals with the manufacture, analytical evaluation, and application of encapsulated products (DeZarn, 1995). Microencapsulation is the process by which small particles of solid, liquid or gas are packaged within a secondary material to form a microcapsule and which allows the user to separate an ingredient from its environment until a point in time when its release is desired (DeZarn, 1995; Augustin et al., 2001).

Microencapsulation was first commercially used in 1954 to create carbonless copy paper (Kondo, 1979). The pharmacological industry has long used encapsulation technology to coat vitamins, minerals, prescription drugs, and over-the-counter drugs, for the purposes of time release, flavor masking, and improved stability within a formulation system (DeZarn, 1995). Similarly, ingredient encapsulation has a number of uses in the food industry such as separate the reactive components within a mixture; mask objectionable flavors; protect unstable ingredients from degradation, such as from heat, moisture, air and light; provide controlled or delayed release; reduce hygroscopicity; and change the physical characteristics of the original material, such as improving its flowability and compression properties, reducing its dustiness, and modifying its density (DeZarn, 1995). However, the food and ingredient industries have only recently been recognized as potentially significant growth areas for the microencapsulation technology and the number of uses and types of microencapsulated ingredients available to the food industry is growing rapidly (Augustin et al., 2001). There is market interest in encapsulated omega-3 fatty acids and phytosterols for cholesterol control, probiotics and prebiotics for gut health, amino acids and proteins as key ingredients in sports and energy foods and drinks, antioxidants which are promoted for general health benefits and vitamins and minerals for fortification of foods (Augustin et al., 2001).

Microencapsulation for food products, however, can pose technically challenging problems, including the selection of the appropriate encapsulation-shell materials from a typically short list of FDA-approved or GRAS (generally recognized as safe) materials and the finding of an encapsulation method that will be economically
feasible in a market sector where profit margins are more dependent upon volume sold than upon the added value of the food product (Brazel, 1999). The size of a microcapsule, generally 1 to 1000 μm in diameter, is an important consideration in the design and selection of materials for targeted delivery and application (Augustin et al., 2001).

Food ingredient encapsulation was once thought to be a rather high priced and custom route to solving unique problems but today, higher production volumes and well-developed technologies have made a number of encapsulated products standard items and available at cost effective prices (DeZarn, 1995).

Coating materials

Once the various release mechanisms and storage or processing needs have been identified, an important step in developing microcapsules is the selection of a coating material that meets necessary criteria, such as mechanical strength, compatibility with the food product, and appropriate thermal or dissolution release and particle size (Brazel, 1999). Most materials are derived from nature, such as polysaccharides (alginate, carrageenan, agarose), proteins (caseinates, gelatin, zein), and fats or fatty acids and their variety allows food producers to select compounds that can be used to encapsulate either hydrophilic solutions or oils, have dissolution-based or melting-based release, and provide a number of textural characteristics (Brazel, 1999). Generally, coating materials composed of one substance have either good barrier or good mechanical properties but not both and different materials therefore are combined: polysaccharides and proteins establish polymer interactions and create a network responsible for the mechanical properties, but they are not efficient water-vapor barriers because of their hydrophilic nature; on the contrary, lipids have a good water-vapor barrier property because of their hydrophobic character, but if a coating material consists of lipids alone, it is usually too brittle (Kester & Fennema, 1986).

Carbohydrates

Carbohydrates are the most commonly used coating materials, especially in flavor encapsulation, due to their ability to absorb volatiles from the environment or to retain them tenaciously (Shahidi & Han, 1993). The two major processes used for encapsulation of food flavorings are spray drying and extrusion (Reineccius, 1991).
Maltodextrins and corn syrup solids, modified starch, cyclodextrins, modified cyclodextrins, sucrose, chitosan and cellulose are some of the carbohydrates used as coating material (Shahidi & Han, 1993). Excellent retention of volatiles has been demonstrated when using modified starch as the encapsulating matrix during spray drying in numerous studies (King et al., 1976; Bangs & Reineccius, 1988; Inglett et al., 1988; Kenyon & Anderson, 1988; Trubiano & Lacourse, 1988). Sucrose has been used for encapsulating food flavors with a process of cocrystallization (Kitson & Sugisawa, 1974; Chen et al., 1988) and mixtures of sucrose and maltodextrins are the most commonly used coatings for extrusion encapsulation. Cellulose has been used in encapsulation of water-soluble food ingredients such as sweeteners and acids, and for enzymes (Desmet et al., 1989).

Gums

Most food gums come from plant materials such as seaweed, seeds, and tree exudates; others are products of microbial biosynthesis; and others are produced by chemical modification of natural polysaccharides (Shahidi & Han, 1993). Gums commonly used as coating materials are seaweed extracts such as alginates, agar and carrageenan (Dziezak, 1991) and have been used to encapsulate high-fat food, flavors and juice (Veliky & Kalab, 1990; Shahidi & Han, 1993). Gum acacia, gum ghatti, gum karaya, and gum tragacanth are referred to as exudates gums with gum acacia being the most commonly used coating material for flavor encapsulation via spray drying (Bhandari et al., 1992; Shahidi & Han, 1993).

Proteins

The most commonly used protein for encapsulating food ingredients is gelatin, which often is used with gum acacia to form coating films by coacervation (Shahidi & Han, 1993). A mixture of protein and carbohydrate has been applied to an encapsulation process of oily substances (Ono & Aoyama, 1979).

Lipids

For many food applications the most important functional characteristic of an edible coating is resistance to migration of moisture and because of their relatively low polarity, the primary function of a lipid coating is generally to block transport of moisture (Kester & Fennema, 1986). Waxes are commonly used as lipid coatings for
encapsulation of food ingredients, especially for encapsulation of water-soluble ingredients (Shahidi & Han, 1993). However, not much is known about their digestibility, which is an important factor if essential food ingredients are to be encapsulated by waxes. An advantage of edible waxes is, that they are significantly more resistant to moisture transport than most other lipids or non-lipid coatings with paraffin wax being the most resistant, followed by beeswax (Schultz et al., 1949; Kamper & Fennema, 1984a; Kamper & Fennema, 1984b). Wax coatings have traditionally been applied to fresh fruits and vegetables to extend postharvest storage life (Kester & Fennema, 1986). In 1980, petroleum wax was allowed to be used under the Federal Food, Drug, and Cosmetic Act in formulating microcapsules for encapsulation of spice flavoring substances in frozen pizza (Food Drug Administration, 1980). While waxes are insoluble in bulk water and do not spread to form a monolayer on the surface due to their unpolarity, triglycerides are insoluble in bulk water as well but they will spread at the interface to form a stable monolayer (Callegarin et al., 1997). Triglycerides' water affinity, or hydrophobicity, depends upon their structure (Callegarin et al., 1997). Long-chain triglycerides are insoluble in water, whereas short-chain molecules are partially water soluble. Above a given concentration, they form aggregates similar to micelles. Fatty acids and fatty alcohol with long chains react similarly and palmitic acid, stearic acid, lauric acid, and stearyl alcohol therefore present efficient moisture-barrier properties when used in edible films. (Callegarin et al., 1997). In general, unsaturated fatty acids are less efficient to control moisture migration because they are more polar than saturated lipids (Hagenmaier & Baker, 1997).

Monoglycerides are insoluble in water, but water is soluble in the hydrophilic part of their structure, thus being used as emulsifiers especially for stabilizing emulsified films (Debeaufort & Voilley, 1995). The water-vapor and oxygen transport through lipids is influenced by the polymorphism of the lipid (α, β', β crystallization) (Kester & Fennema, 1989b; Kester & Fennema, 1989a) and by the temperature producing a change in the physical state and in the structure of the coating lipid (Callegarin et al., 1997). Acetylated monoglycerides, called acetoglycerides, have a characteristic flexible, wax-like solid state with a water-permeability considerably lower than that of most polysaccharide films, but greater than that of ethyl- and methyl-cellulose (Lovegren & Feuge, 1954; Kanig & Goodman, 1962) and is often used in edible film

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formulations to coat frozen food because of its plasticizer characteristics (Kester & Fennema, 1986; Stuchell & Krochta, 1995). Lecithin has been used for encapsulation of lysozyme and pepsin (Koide & Karel, 1987). Blended with other coating materials, lecithin is able to change the structure of formed microcapsules and a mixture of lecithin and polyethylene has been used to encapsulate sweeteners and flavor compounds (Shahidi & Han, 1993). The lipids most used are fatty acids with a number of carbon atoms between 14 and 18, mono-, di- and tristearin, stearyl alcohol, hydrogenated and nonhydrogenated vegetable oils, and waxes (beeswax, candelilla, paraffin) (Callegarin et al., 1997).

Liposomes, defined as a structure compound of lipid bilayers, that enclose a number of aqueous or liquid compartments are usually prepared from lecithin and cholesterol and have been studied extensively over the past 30 years (Shahidi & Han, 1993). Recently, due to the health concern of cholesterol, other lipids have been evaluated for the preparation of liposomes and liposomes prepared from lecithin and stearic acid were similarly stable compared to liposomes prepared with lecithin and cholesterol (Hsieh et al., 2002). Virtually any substance, regardless of solubility, electrical charge, molecular size, or other structural characteristics, can be incorporated into liposomes, provided that the substance does not interfere with liposome formation (Gregoriadis, 1987). Water-soluble materials may be entrapped in the aqueous phase of liposomes, whereas lipid-soluble materials will be incorporated into the lipid phase. A limitation of the use of liposomes in some foods may be their lack of stability in the presence of moderate levels of oils or hydrophobic proteins (Kim & Baianu, 1991).

**Encapsulation techniques**

The process of microencapsulation involves: 1) formation of a coating around the core material; 2) keeping the core material inside the coating, so that it does not escape and preventing the entrance of undesirable materials that may harm the core; 3) release of the core material at the right time and at a controlled release rate (Shahidi & Han, 1993). The various techniques for encapsulation can be divided into two groups according to the resulting capsule type. Spray drying, spray chilling or spray cooling, fluidized bed coating and rotational suspension separation are typically referred to as 'matrix' encapsulation because the particles are more adequately
described as aggregates of active ingredient particles buried in the fat matrix, while 'true' encapsulation such as extrusion coating, liposome entrapment, coacervation, inclusion complexation, and centrifugal extrusion is usually reserved for processes leading to a core/shell type of microcapsules (Dziezak, 1988; Gibbs et al., 1999; Gouin, 2004). A matrix encapsulation process leaves a significant proportion of the active ingredient lying on the surface of the microcapsules or sticking out of the fat matrix, thus having direct access to the environment resulting in faster release compared to a core/shell type of capsule.

**Spray drying**

Traditionally, spray drying has been the most common method of encapsulating food ingredients and still is one of the most cost-effective and widely used method (Risch, 1995). It has most commonly been used to provide flavor oils with some protection against degradation/oxidation and to convert liquids to powders (Gouin, 2004). For this process the coating material (maltodextrin, modified starch, gum acacia or a combination thereof) is hydrated and the core material added and homogenously mixed (Risch, 1995). The mixture is fed into a spray dryer where it is atomized through a nozzle or spinning wheel. Hot air flowing in either a co-current or counter-current direction contacts the atomized particles and evaporates the water, producing a dried particle that is a carrier matrix containing small droplets of the core. The particles are collected at the bottom of the dryer. This process results in a very fine powder which needs further processing such as agglomeration to instantize the dried material or make it more readily soluble if it is for a liquid application (Risch, 1995). Since almost all spray drying processes in the food industry are carried out from aqueous feed formulations, the coating material must be soluble in water at an acceptable level and the number of such material is limited (Gouin, 2004). Another limitation is that due to the heat required for evaporation of water from the system, spray drying is not feasible for heat sensitive materials (Risch, 1995). Zilberboim et al. (1986b; 1986a) have developed an alternative process for heat labile material in which the mixture is atomized into ethanol which acts as a dehydrating liquid and microcapsules are then separated from the solution by filtration and dried in a vacuum oven at low temperature. However, the batch wise production makes this process much more costly than spray drying.
Literature Review

Spray cooling/chilling
The problems of flavor loss during the 'drying' stage of spray drying, and the generally low flavor levels attainable by this process, have led to the investigation of a number of alternative methods such as spray chilling and spray cooling (Arshady, 1993). These methods are similar to spray drying in that core material is dispersed in a liquefied coating material and atomized with the difference that there is generally no water to be evaporated (Risch, 1995). The mixture is atomized into either cool or chilled air, which causes the coating material to solidify. The coating material is typically a fractionated or hydrogenated vegetable oil with a melting point between 32-42°C in spray chilling and between 45-122°C for spray cooling (Risch, 1995). These two methods are most often used to encapsulate solid materials such as vitamins, minerals or acidulants (Risch, 1995) and to convert liquid hydrophilic ingredients into free flowing powders (Gouin, 2004). Since the coating material consists of a fat, microcapsules can be expected to be more resistant to water-vapor than microcapsules produced by spray drying. This process is the least expensive encapsulation technology (Gouin, 2004).

Extrusion coating
Extrusion involves dispersion of the core material in a molten carbohydrate mass which is then forced through a die into a dehydrating liquid such as isopropyl alcohol which hardens the coating to trap the core material (Risch, 1995). The filaments are then broken into small pieces, separated and dried. This process has almost exclusively been used for the encapsulation of volatile and unstable flavors in gassy carbohydrate matrices (Reineccius, 1991; Blake, 1994; Gunning et al., 1999). It provides true encapsulation because all residual core material is removed from the surface when the material contacts the dehydrating liquid resulting in products with an excellent shelf life (Risch, 1988). However, the particles formed by this process are rather large with 500-1000 μm and limit the use in application where mouthfeel is a crucial factor (Gouin, 2004).

Fluidized bed coating
Another technique finding application in the food industry is fluidized bed coating, also referred to as air suspension coating or the Wurster process, which is typically used to coat solid particles (Risch, 1995). In this process, the particles to be coated
are circulated through a chamber with high velocity air and the coating material is atomized into the particle stream and deposited on the surface. This technology is one of the few advanced technologies capable of coating particles with basically any kind of coating material (Gouin, 2004). A number of food ingredients have been encapsulated by this process, such as ascorbic acid (Knezevic et al., 1998), acidulants for processed meat (Weiss & Reynolds, 1989) and leavening agents (Balassa & Brody, 1968). Spray dried particles, such as spray-dried flavor microcapsules can also be further coated by fluidized bed, with a fat layer, for instance, to impart better protection and shelf life (Gouin, 2004). The use of melted fats, waxes or emulsifiers as coating material is less expensive since no evaporation needs to be done (Gouin, 2004).

**Liposome entrapment**

Today, liposomes can be produced continuously and on a large scale with the large unilamellar vesicles (≈ 1 μm) being the most appropriate liposomes for the food industry because of their high encapsulation efficiency, their simple production methods and good stability over time (Gouin, 2004). Liposomes are capable of delivering both lipophilic and aqueous-based active materials (Reineccius, 1995). The only materials which cannot be included in the liposome are substances which are insoluble in either lipid or aqueous phases or those which have significant solubility in both phases such as most flavor compounds. Liposomes are generally produced by dispersing the lipid formulation in an aqueous solvent at which time the liposomes are formed and by purifying or separating the liposomes from the bulk liquid (Reineccius, 1995). Lipid soluble core materials are generally included in the liposome by adding them to the initial lipid mixture while the aqueous soluble materials are added in different ways depending on the methods used (Reineccius, 1995). The great advantage of liposomes over other microencapsulation technologies is the stability liposomes impart to water-soluble material in high water activity application (Gouin, 2004).

**Coacervation**

Coacervation, the process patented for carbonless paper, consists in the separation of a liquid phase of the coating material from a polymeric solution and the surrounding of the suspended core material (Risch, 1995). The coating is then
solidified. It is typically used to encapsulate flavor oils, but had also been used (Korus et al., 2003) for fish oils (Lamprecht et al., 2001) and vitamins (Junyaprasert et al., 2001). However, to commercialize a coacervated food ingredient is very expensive and rather complex (Gouin, 2004).

**Inclusion complexation**

Inclusion complexation is the only method that takes place on a molecular level. Cyclodextrins, typically β-cyclodextrin, which have a hollow, hydrophobic center with a hydrophilic outer surface are used for this process (Risch, 1995). In solution, less polar molecules will replace the water molecule in the center of the cyclodextrin thereby becoming less soluble and precipitating out of the solution. Drawbacks include the low payload and the rather high cost of the cyclodextrins (Gouin, 2004).

**Centrifugal extrusion**

This process is a liquid coextrusion process in which a liquid core material is pumped through the inner orifice and a liquid coating material through the outer orifice forming a coextruded rod of core material surrounded by shell material which is broken by the rotation of the device forming the capsules (Schlameus, 1995). Depending on the coating material capsules are collected in a liquid reaction bath, by simple cooling, by powder collection, misting or solvent evaporation. Capsule sizes of 150-2000 μm can be reached and a wide variety of coating material can be used (Schlameus, 1995).

**Rotational suspension separation**

Rotational suspension separation involves the suspension of the core material in the selected coating material and the introduction of this mixture onto a rotating disk. The encapsulated particles are spun off the disk and then dried or chilled (Sparks et al., 1995). It is a continuous process and can coat particles from roughly 30 μm up to several millimeters at high production rates using a wide variety of coating materials such as solutions, suspensions or meltable materials including fats, waxes and diglycerides.

Although many different encapsulation techniques and coating materials exist, there are large differences in cost when producing microcapsules. Popplewell et al. (2001)
have developed a model to compare specific encapsulation options to determine whether they economically fit targeted applications.

**Encapsulation of food ingredients**

The history of microencapsulation in the food industry dates back to at least 1951, when Griffin patented a process for the preparation of solid oil concentrates (Griffin, 1951). Since then various food ingredients have been microencapsulated and several processes have been patented.

**Sweeteners**

Numerous attempts have been made to stabilize aspartame which can be degraded by heat in chewing gums as well as in other food products. In one of the several patents, the aspartame is encapsulated in a material such as ethylcellulose or methylcellulose protecting the aspartame during baking so that the finished goods retain the desired sweetness level (Redding et al., 1992). Another patent discloses the use of a matrix of lecithins, fatty acids and waxes, glycerides and a silicon-based antifoam agent to encapsulate the sweetener by spray cooling (Bodor & Dokuzovic, 1992). Such microcapsules show increased temperature tolerance.

**Flavors**

Microencapsulation provides a means of improving flavor in foods. Since many dried flavors contain volatile components microencapsulation can protect them during production and allows controlled release at the appropriate time (Mothes, 1997; Benczedi & Blake, 1999). For example, encapsulated flavors in chewing gums are released on chewing (Gudas et al., 1998; Pszczola, 1998) and capsules of frozen flavor which are entrapped in solidified oil in refrigerated desserts are released when the oil liquefies in the mouth (Graf & Van Leersum, 1996).

Kim and Morr (1996) evaluated the encapsulation properties of several commercial food proteins and gum arabic in microcapsules containing a liquid orange oil emulsions produced by spray drying. Soy protein isolate was most effective in retaining orange oil during spray-drying and orange oil in this shell was most stable against oxidation. In a recent study the stability of single encapsulated limonene produced by spray-drying using a mixture of maltodextrin and different gums was
compared to that of double encapsulated limonene, additionally coated in a second step with hydrogenated palm kernel oil by fluidized bed coating (Cho & Park, 2002). Double encapsulation resulted in a two times larger mean particle size, a reduction of moisture uptake and increased oxidation stability demonstrating the barrier effect of the secondary-fat coating. Similar results were obtained by Onwulata et al. (1998) comparing the stability of single and double encapsulated butter oil powders, encapsulated with granulated sucrose and in a second coating step with a mixture of vegetable waxes. A patent describes the process of liposome encapsulated ingredients, such as flavors, flavor enhancers, preservatives, anti-staling agents, sweeteners and other labile food additives for the use of cookie production (Lengerich et al., 1991). The core material may be released during baking, storage or eating. Another patent describes the encapsulation of flavoring agents by coacervation and the use of such microcapsules in chewing gum and confectionary preparations (Cherukuri et al., 1993).

Oils

Omega-3 polyunsaturated fatty acids (PUFA) have been identified as essential to human subjects during the whole of their lifetime and enrichment of food with fish oil has been studied intensively (Schrooyen et al., 2001).

A liposome patent describes the encapsulation of a highly unsaturated lipid such as an unsaturated triglyceride omega-3-fatty-acid-containing fish oil for incorporation into margarine (Haynes et al., 1992). The liposomes have been shown to stabilize effectively the readily oxidizable lipids and to provide extended shelf life. Heinzelmann et al. (1999; 2000) showed in their study that fish oil can be encapsulated in a carbohydrate matrix together with antioxidants by freeze-drying resulting in a product with good oxidation stability. Similarly, a study by Baik et al. (2004) demonstrated prolonged oxidative stability of encapsulated fish oil when α-tocopherol was added. Kolanowski et al. (1999) studied the possibilities of enriching certain food products with encapsulated n-3 PUFA and found that instant powder-milk-based formulae concentrates, fats and products of high sweetness and flavor intensity were most suitable for PUFA enrichment. A more recent study conducted by the same author showed that spray-dried fish oil microcapsules coated with methylcellulose have improved stability (Kolanowski et al., 2004). Another study showed that yogurts, fermented milks and processed cheese fortified with tuna oil
encapsulated with processed milk protein-carbohydrate films have higher sensory scores than those fortified with an equivalent amount of nonencapsulated oil (Sharma et al., 2003). Bioavailability studies with encapsulated fish oil in a milkshake or in bread, biscuit and soup have shown to increase plasma n-3 fatty acids as effectively as a fish oil supplement (Higgins et al., 1999; Wallace et al., 2000). Another study showed that consumption of bread containing microencapsulated tuna oil significantly increased plasma long chain n-3 PUFA levels compared to baseline (Yep et al., 2002). Other oils which were successfully encapsulated include wheatgerm and evening primrose oil encapsulated in sodium alginate (Chan et al., 2000), linoleic acid encapsulated in soluble soybean polysaccharide by spray drying (Fang et al., 2003) and arachidonic acid and arachidonoyl ascorbate microencapsulated with gum arabic or soluble soybean polysaccharide by spray drying (Watanabe et al., 2004).

Enzymes
Work performed by Magee et al. (1981; 1981) showed that milk-fat coated capsules containing a multi-enzyme system can be produced and are efficacious and predictable in enhancing and regulating flavor development in cheese. Results showed that the capsules supplied a controlled micro-environment for maximum interaction between the heterogenous mixture of enzymes, co-factors, and substrates generating acetoin and diacetyl reaching eight times the concentration compared to control cheeses. Similar results were obtained by encapsulation of the enzymes in liposomes when added to cheddar cheese (Law & King, 1985; Kirby et al., 1987; Kirby & Law, 1987).

In order to help people not able to digest lactose, liposomes containing β-galactosidase were developed and stored in milk at 4°C for 20 days (Rao et al., 1995). Lactose was decreased by 20% in milk containing liposomes produced by reverse-phase-evaporation and enzyme activity in the microcapsule did not change during the storage period. In vitro digestion of lactose showed a significant decrease in lactose content of milk containing encapsulated β-galactosidase. A more recent study encapsulated β-galactosidase in medium-chain triacylglycerol or polyglycerol monostearate and showed no change in lactose content in milk up to 12 days of storage and no increase in sweetness (Kwak et al., 2001).
Vitamins and minerals

Fat soluble vitamins

Both lipid-soluble and water-soluble vitamins can be encapsulated to extend shelf-life using various technologies (Schrooyen et al., 2001). Lipid-soluble vitamins are much easier to encapsulate than water-soluble ingredients and a commonly used procedure is spray drying of emulsions (Schrooyen et al., 2001). A study encapsulating carrot coagulum with various dextrose equivalent (DE) hydrolyzed starches by spray drying showed that hydrolyzed starch with the highest DE of 36.5 results in the highest retention of \(\alpha\)- and \(\beta\)-carotene with a half-life of 450 days at 21°C (Wagner & Warthesen, 1995). Air was critical in carotene stability, but exposure of encapsulated carrot powders to light did not accelerate degradation. In another study, \(\beta\)-carotene was encapsulated in 25 DE maltodextrin using spray drying, freeze drying and drum drying and its stability during storage at different temperature and humidity was determined (Desobry et al., 1997). Losses during encapsulation were between 8-14 % for the three encapsulation methods used. After storage for 15 weeks the drum-dried product showed the highest \(\beta\)-carotene retention with \(\approx\) 40% of the initial content, followed by the freeze-dried product with \(\approx\) 35% and the spray dried product with a remaining content of < 30% indicating problems for the addition of a product with a short shelf life to food. A study conducted by Desobry et al. (1999) encapsulated \(\beta\)-carotene in mixtures of 4 DE- and 15-DE-maltodextrin with either glucose, galactose or lactose using different composition but having the same final DE of 25 by spray drying. Spray drying led to \(\approx\) 11% degradation of \(\beta\)-carotene during processing for all samples. The maltodextrin 4 DE mixtures showed better retention during storage than the others and had less \(\beta\)-carotene on the surface of the capsule but were more hygroscopic.

A recent study produced microcapsules containing water and oil soluble carotenoids by spray-drying of water-in-oil-in-water multiple emulsions stabilized by a blend of biopolymers (Rodriguez-Huezo et al., 2004). Microcapsules obtained by spray drying of multiple emulsions with high solid contents showed the best morphology, highest microencapsulation efficiency, and highest total carotenoids retention. Vitamin A fortification of monosodium glutamate (MSG) using vitamin A palmitate beadlets (250CWS) from Hoffmann La Roche (Basel, Switzerland) has been used in
the Philippines but the small round beadlets segregated from the larger MSG crystals and caused discoloration (Hall, 1995). Therefore, vitamin A beads were whitened with titanium dioxide (TiO$_2$) and a fine powdered form of MSG was added which adhered to the bead approaching more closely the particle size of MSG. A field trial in Indonesia using the whitened MSG showed a marked decrease in the incidence of eye disease and a significant decrease in child mortality (Muhilal et al., 1988a). However, large scale distribution showed color problems in certain areas and higher pigment levels (TiO$_2$) were necessary which was achieved by fluidized bed coating (Hall, 1995). However, field trials showed that this product had not the necessary chemical stability of vitamin A most probably due to high ambient temperature and humidity in Indonesia. A new coating with a good moisture barrier was developed and has shown visual and chemical stability (Hall, 1995) but field trial results are not available.

In a work done by Chen and Wagner (2004) encapsulated nanoparticles containing vitamin E in a starch matrix were produced by spray drying and have shown improved turbidity and physical stability in beverages compared to nonencapsulated products. Vitamin D encapsulated in liposomes has been incorporated into cheese curd at a high efficiency but losses during storage were up to 40% in 7 months and were higher than from cheese containing nonencapsulated vitamin D (Banville et al., 2000).

Water-soluble vitamins

It is a true challenge to encapsulate water-soluble food ingredients to prevent these compounds from leaking away into the food system and the most obvious way is to encapsulate them by spray cooling and spray chilling using fat or wax as coating material (Schrooyen et al., 2001). However, for application in liquid food systems, the best way is to produce liposomes (Schrooyen et al., 2001). Ascorbic acid was encapsulated with high efficiency inside liposomes and storage tests showed increased stability in aqueous solutions and in the presence of common food compared to nonencapsulated ascorbic acid (Kirby et al., 1991). Authors suggest that these liposomes could have a potential for the preservation of nutritional supplements of vitamin C in solution and for the development of naturally derived antioxidant systems for food protection. Another study used fluidized bed coating and hydrophobic coating materials to encapsulate ascorbic acid and showed a delayed
release of the core in water compared to free ascorbic acid (Knezevic et al., 1998). In contrast, a study using gum arabic or rice starch as coating material to produce spray dried microcapsules showed no increase in stability compared to free crystalline ascorbic acid (Trindade & Grosso, 2000). Uddin et al. (2001) encapsulated ascorbic acid using different encapsulation methods and showed that microencapsulation could prevent the ascorbic acid color change, retard its core release rate, and generally mask its acid taste. Recent studies encapsulated ascorbic acid with medium-chain triacylglycerol or polyacylglycerol monostearate and microcapsules were used to fortify milk together with encapsulated ferric ammonium sulfate (Lee et al., 2003a; Lee et al., 2004). Milk fat oxidation was prevented by the addition of either encapsulated or nonencapsulated ascorbic acid up to 8 days of storage at 4°C and the sensory quality of the milk was shown to be well maintained by the encapsulation of both L-ascorbic acid and iron. A human study showed a significant increase in serum Fe concentration and transferrin saturation in subjects consuming milk containing both encapsulated Fe and encapsulated ascorbic acid, compared to those consuming nonencapsulated Fe or encapsulated Fe fortified milk without ascorbic acid (Lee et al., 2003b).

Dib Taxi et al. (2003) encapsulated the juice of the camu-camu fruit, an Amazonian fruit with a high vitamin C content, with maltodextrin and gum arabic by spray drying and found a vitamin C retention of ≈ 6%. However, information about the stability of the capsule during storage or when added to food are not given. Reineccius et al. (1995) have shown that liposomes containing ascorbic acid and vitamin E together can have a synergistic antioxidant effect and could be used for protection of emulsion-type foods.

**Minerals**

The effect of magnesium oxide encapsulated with modified cellulose (Fortitech Inc., Schenectady, USA) on growth and bone minerals was assessed in rats. Results suggest that encapsulation with modified cellulose does not alter magnesium bioavailability, though addition of high levels of either encapsulated or nonencapsulated magnesium decreases food intake and growth and modifies calcium metabolism. However, they also showed that it is possible that encapsulation could to some extent minimize the adverse effects of excess magnesium on calcium homeostasis.
Encapsulation of calcium lactate in a lecithin liposome has been successful in fortifying soy milk reaching levels equivalent to those in normal cow's milk and the fortified milk remained stable for at least 1 week when stored at 4°C (Hirotsuka et al., 1984).

Encapsulated iron in food fortification

Since Fe catalysis oxidative processes in fatty acids, vitamins and amino acids, and consequently alters sensory characteristics and decreases the nutritional value of the food, microencapsulation can be used to prevent these reactions, although bioavailability should be checked carefully (Schrooyen et al., 2001). The main advantage of Fe encapsulation is that it should allow the addition of Fe compounds of high relative bioavailability to difficult food vehicles, such as cereal flours and low-grade salt, without causing the customary color and flavor changes (Hurrell et al., 2004). Commonly used coatings are hydrogenated palm oil or soybean oil which protect against moisture but melt during cooking and microencapsulated Fe compounds are usually manufactured by fluidized bed coating or spray chilling (Hurrell et al., 2004). A coating material to Fe ratio close to 1:1 has been shown not to change the bioavailability of FeSO₄ in rats and to improve Fe status in humans (Hurrell et al., 1989a; Zimmermann et al., 2003b). However, greater amounts of coating material or the inclusion of waxes may decrease bioavailability (Hurrell et al., 2004). Encapsulated Fe compounds are generally suitable for the fortification of most dry products such as infant formulas and cereals but are added to other food stuffs as well (Zimmermann, 2004).

Ferrous sulfate

Several studies have shown good stability of ferrous sulfate encapsulated in hydrogenated soybean or palm oil, mono-and diglycerides, maltodextrin or ethyl cellulose during storage when added to wheat or rice flour not causing fat oxidation or off-flavor (Harrison et al., 1976; Hurrell, 1985; Hurrell et al., 1989a). However, when hot water was added during preparation, off-color and bad odor developed.

If fluid food products are to be fortified with Fe, liposome technology is the method of choice (Schrooyen et al., 2001). Several attempts have been made to fortify fluid milk and other dairy products with Fe. Boccio et al. (1995) added ferrous sulfate
encapsulated with soy lecithin (SFE-171) to fluid milk and compared its bioavailability with that of FeSO₄ in mice. These results demonstrated a significantly higher bioavailability of the encapsulated compared to the nonencapsulated compound and that the addition of cacao or mate tea had no influence on its absorption but other additives like tea and coffee decreased its absorption. Further studies in mice conducted by the same authors using the same SFE-171 Fe compound showed that heat treatment and storage for 6 months did not result in decreased Fe bioavailability of the Fe-fortified milk and that absorption from the encapsulated compound was higher compared to that from FeSO₄ in milk and comparable to that of ferrous sulfate in water and ferrous ascorbate in water, both of them considered as reference standards (Boccio et al., 1996; 1997; 1998). These studies also showed that the Fe from the SFE-171 follows the same metabolic pathway as the Fe from the reference standards and the authors suggested that encapsulation of the Fe improved its bioavailability by reducing its interaction with protein, calcium and other milk components. A similar study was done in rats showing a bioavailability of 1-1.2 fold that of ferrous sulfate (Zubillaga et al., 1996). Another study estimating the bioavailability of SFE-171 using the prophylactic-preventive method in rats and comparing it to ferrous sulfate and elemental Fe confirmed this result (Lysionek et al., 2000). Relative bioavailability values were 98% and 34% for the SFE-171 and elemental Fe, respectively. Two human studies in iron replete adults using radioisotopes have shown a bioavailability of 9-10 % (Gotelli et al., 1996; Uicich et al., 1999).

The same SFE-171 was used to fortify cheese (Lysionek et al., 2002). Petit-Suisse Cheese was fortified with microencapsulated FeSO₄ (SFE-171), ferric orthophosphate and FeSO₄ and their bioavailability tested in rats using the prophylactic assay. Bioavailability of the two ferrous sulfate compounds was equal and significantly higher compared to that of ferric orthophosphate suggesting that SFE-171 was stable through industrial processing.

Another food vehicle fortified with FeSO₄ encapsulated in partially hydrogenated vegetable oil is salt (Zimmermann et al., 2003b). Salt dual fortified with this Fe compound and iodine showed good iodine stability, no change in color, odor, and/or taste in the foods containing fortified salt and high efficacy in improving the iron status of school aged children in Morocco. However, during the damp season, when
the moisture content of the local salt is high (≈ 3%), the salt developed a mild yellow color during storage.

Ferrous fumarate
Ferrous fumarate is another good bioavailable iron compound which due to its insolubility in water is likely to cause less organoleptic changes compared to ferrous sulfate. Some attempts have been made to encapsulate this compound. In a process patented by Mehansho et al. (1999) ferrous fumarate and ferrous succinate were encapsulated in liposomes that may be used for fortification of beverages while minimizing off-flavors and interactions with other components in the food. Zlotkin et al. (2001) used ferrous fumarate encapsulated in hydrogenated soybean oil plus ascorbic acid as “sprinkles” to be mixed into complementary food in anemic Ghanaian children 6–18 months old. The range of potentially absorbed Fe from the sprinkles was similar to that from ferrous sulfate drops and 58% and 56%, respectively, improved from an anemic to a non-anemic state. Sprinkles added to food did not change the color or texture of the food and the taste was not affected. In another study using the double stable isotope technique the absorption of sprinkles containing encapsulated ferrous fumarate or non encapsulated ferrous fumarate mixed into rice-based and wheat-based complementary foods was tested in 7-12 months old non-anemic infants (Liyanage & Zlotkin, 2002). This study showed a significantly lower absorption of encapsulated ferrous fumarate compared with non encapsulated ferrous fumarate, although the absorption from a commercial encapsulated product can be expected to be higher, due to a lower coating:core ratio. A recent study tested sprinkles containing encapsulated ferrous fumarate plus ascorbic acid added to a maize-based porridge in infants with IDA, ID or sufficient Fe status using stable isotopes (Tondeur et al., 2004). Mean Fe absorption ranged from 4.5 (iron sufficient and ID) to 8.3% (IDA) indicating that sprinkles fortified with encapsulated ferrous fumarate and ascorbic acid and added to complementary foods would probably provide adequate iron.

Other iron compounds
An approach to fortify fluid milk was applied by Kwak et al. (2003) using ferric ammonium sulfate as Fe source and polyglycerol monostearate as coating material. Results showed significantly better performance of organoleptic properties of milk
containing the encapsulated compound compared to milk containing the nonencapsulated compound and faster lipid oxidation in milk with nonencapsulated Fe than in milk with encapsulated Fe. Studies on cheese fortification were performed by Jackson and Lee (1991) by producing microcapsules containing FeCl₃ or FeSO₄ plus ascorbic acid coated with hydrogenated milk fat or stearine and results showed that stearine-coated iron salts were the most stable compounds causing less oxidation of fat in cheese compared to those fortified with unprotected Fe salt (Jackson & Lee, 1992).

As demonstrated above, encapsulated ferrous sulfate and encapsulated ferrous fumarate have the potential to be used in food fortification and were recently recommended by the Pan American Health Organization (PAHO/WHO, 2002) in wheat and maize flours provided that manufacturing and cooking practices do not result in unacceptable organoleptic change to foods if the capsule material melts (Hurrell et al., 2004). However, the cost-effectiveness of fortification with encapsulated Fe compounds remains to be determined. Current cost estimates for encapsulated ferrous sulfate are four to seven times that of non encapsulated ferrous sulfate, but the price could fall if produced in large-scale (Zimmermann, 2004).

Another approach to fortify difficult to fortify food vehicles besides encapsulation of good bioavailable compounds could be the use of poorly soluble Fe compounds such as FePP, which do not need to be encapsulated and provoke less sensory changes but at the same time are more expensive than ferrous sulfate and need to be added in higher quantities due to their lower bioavailability which might be overcome by particle size reduction.
FERRIC PYROPHOSPHATE

Properties

Ferric pyrophosphate (iron (III) pyrophosphate, \( \text{Fe}_4(\text{P}_2\text{O}_{7})_3 \times \text{H}_2\text{O} \)) is a tan or yellowish white colorless powder (Food and Drug Administration, 2001), is odorless and has a slight Fe taste (The Japan Food Chemical Research Foundation, 2005). It is prepared by reacting sodium pyrophosphate with ferric citrate (Food and Drug Administration, 2001) or ferric chloride (Tsuchita et al., 1991). The powder consists of \( \text{Fe}_4(\text{P}_2\text{O}_{7})_3 \times 9 \text{H}_2\text{O} \) if dried at 110°C and is soluble in mineral acids and \( \text{NH}_3 \)-solution and insoluble in acetic acid, \( \text{H}_2\text{SO}_4 \) and \( \text{NH}_4\text{Cl} \)-solution (Gürs, 1997). Its solubility in water is very low with 0.0050 Mol/L at 25°C and 0.0082 Mol/L at 50°C (Gürs, 1997). Several studies have tried to identify the crystal structure of FePP. One study, performed by Elbouaanani et al. (2002) looked at the crystallographic structure of \( \text{Fe}_4(\text{P}_2\text{O}_{7})_3 \). The three-dimensional framework was found to be built up from \( \text{Fe}_2\text{O}_9 \) clusters of two face-sharing octahedra, linked by bent diphosphates \( \text{P}_2\text{O}_7 \) (P-O-P \(~156°\)). A study conducted by d'Yvoire (1962) has shown that \( \text{FeHP}_2\text{O}_7 \) looses most of the water when heated to 600°C resulting in a amorphous product and that above 650°C the product starts to crystallize resulting in a mixture of neutral diphosphate \( \text{Fe}_4(\text{P}_2\text{O}_{7})_3 \) and long chain polyphosphate \( \text{Fe}(\text{PO}_3)_3 \).

The Fe content of commercial regular FePP is \(~25\%\) (Dr. Paul Lohmann GmbH, Emmerthal, Germany; ScienceLab, Kingwood, Texas, USA; Shanghai United Food Additives Co., Ltd., Shanghai, China). Regular FePP is used in food as a nutrient supplement with no limitation other than current good manufacturing practice and it may also be used in infant formula (Food and Drug Administration, 2001).

More recently, different forms of FePP have been developed. Besides the regular FePP, a micronized form was developed by Dr. Paul Lohmann GmbH (Emmerthal, Germany). This product is produced by conventional grinding, contains no additives, has an average particle size of 2.5 \( \mu \text{m} \) and a Fe content of 21 to 25\% and is not patented (Dr. Paul Lohmann GmbH, Emmerthal, Germany).

Another commercially available but patented form of FePP is SunActive Fe (Taiyo Kagaku Co., Ltd., Yokkaichi, Mie, Japan), which is composed of micronized FePP (average particle size 0.3 – 0.5 \( \mu \text{m} \)) and is stabilized and protected using unique emulsifiers (Wade, 2004). Products with SunActive Fe are generally recognized as
safe (GRAS) and contain maltodextrin, FePP, polyglycerol esters and hydrolyzed lecithin as ingredients (Wade, 2004). SunActive Fe is available as a liquid form (superdispersed) as SunActive Fe™ (12% Fe, average particle size 0.3 µm) and as a powder as SunActive Fe-P80 (8% Fe, average particle size 0.5 µm) (Taiyo Kagaku Co., Ltd., Yokkaichi, Mie, Japan). In solution, SunActive Fe appears clear to slightly translucent, depending on dosage and remains in suspension in liquid products being stable against heat, salt, pH and oxidation and can therefore be used for the fortification of dairy-based products, such as milk, drinkable yogurts and soymilk (Wade, 2004; Taiyo Kagaku, 2005).

Another emulsified form of FePP is Lipofer® (Lipofoods S.L., Barcelona, Spain). Lipofer® is a liposome-type complex that contains a FePP salt (Lipofoods S.L., 1998). Two types exist, Lipofer® dispersible (ingredients: starch, FePP (8% Fe), lecithin) which is a fine free-flowing powder, easy to disperse in foods like bakery products, cereal bars or powdered drink and Lipofer® liquid (ingredients: water, FePP (12% Fe), lecithin, sodium chloride, carboxymethylcellulose) which is a suspension of very fine particles that do not sediment in beverages and therefore can be used in drinks as well as in dairy products like yogurts. The color of both products is white to light yellow. The company states that Lipofer® does not interact with the rest of components of the digestion avoiding inhibitory effects and absorption in mice has been shown to be significantly higher compared to that from ferrous fumarate. The difference to SunActive Fe lies in the composition and the average particle size, which is 7 µm compared to 0.3 µm for the SunActive Fe form (Kloots et al., 2004).

**Bioavailability**

In general, Fe compounds such as the pyrophosphates, orthophosphates, hydroxides, and oxides are relatively insoluble in water, compared to the sulfates and chlorides (Tsuchita et al., 1991). These insoluble salts show relatively low bioavailability but are often the only possibility to be used for the fortification of several difficult to fortify food vehicles. Several in vitro, animal and human studies have looked at the bioavailability of FePP.
In vitro studies

A recent study evaluated the fortification of whole-grain wheat flour chapatis with different sources of Fe including FePP in vitro by measuring the amount of dialyzable Fe after simulated gastrointestinal digestion and subsequent intestinal absorption of the released Fe using Caco-2 cell layers (Kloots et al., 2004). Although the dialyzability of FePP was expected to be less than that of ferrous sulfate as reported in an earlier study (Davidsson et al., 2000) similar dialyzability of chapatis fortified with FePP and FeSO₄ was demonstrated. The Caco-2 absorption as well was not different comparing FePP and FeSO₄ fortified chapatis. However, these results demonstrate that dialyzability and Caco-2 measures have limited power to predict absorption of iron compounds in humans.

Animal studies

A study conducted in anemic chicks and rats using the hemoglobin repletion technique showed a relative bioavailability (RBV) of FePP of 45% of ferrous sulfate (Fritz et al., 1970). A recent rat study showed a slightly higher RBV of 56% for regular FePP (Sakaguchi et al., 2004). This value was confirmed by another rat study demonstrating a RBV of 58% for FePP when added to the diet in powder form and showing an increase in the RBV to 83 and 103% that of ferrous sulfate when FePP was solubilized with sodium citrate or ammonium citrate, respectively (Hurrell et al., 1989a). Earlier, Pla et al. (Pla et al., 1973) had similarly reported an RBV of 96% for sodium citrate-stabilized ferric pyrophosphate.

Human studies

Measuring the bioavailability of FePP has for a long time not been satisfactory in humans, partly because of the difficulty in obtaining a radioactive salt of the same specifications as the commercial product and results of the first studies showed widely varying values. In an early human radioisotope study, salt fortified with labeled FePP was administered with water to healthy male Indian adults and the absorption estimated by whole body counting was found to be negligible (Narasinga Rao et al., 1972). Another early human study tried to measure the absorption of FePP from an infant cereal by adding an extrinsic tag of radioactive FePP and found a similar absorption as from ferrous sulfate, but it is not clear whether the extrinsic tag and the fortification Fe were of the same specifications (Derman et al., 1980).
Three more recent human radioisotope studies with FePP fortified foods were carried out in adults and have found RBVs of 39% and 15% compared to ferrous sulfate from wheat-based infant cereals fortified with FePP (Hurrell et al., 1989a; 2000) and of 75% when FePP was added to a chocolate drink immediately before consumption (Hurrell et al., 1991).

A more recent stable isotope study in infants looking at the absorption from FePP fortified wheat-soy infant cereal found that FePP was only one-third as well absorbed as ferrous fumarate (Davidsson et al., 2000). The absorption of FePP was not directly compared to ferrous sulfate. A recent stable isotope study in adults showed an RBV of 36% for an infant cereal fortified with FePP compared to ferrous sulfate (Fidler et al., 2004a).

Although the absorption from FePP is generally relatively low, an efficacy study in Pakistani infants fed a wheat-milk complementary food fortified with FePP or ferrous fumarate as part of their normal diet, has shown that the Fe status of the two fortified groups was not different after 12 months but that it was significantly higher than the control group receiving no Fe (Javaid et al., 1991). However, about half of the study infants were still anemic after the 12 months study period indicating that the Fe dose was likely to be too low.

**Influence of processing and physicochemical properties**

An important consideration with FePP is that bioavailability may change during food processing and several studies looked at this effect. Two studies looked at the effect of processing on availability of FePP in a soy isolate infant formula (Theuer et al., 1971) and a liquid milk-based formula (Theuer et al., 1973) and demonstrated that sterilization increased the relative Fe availability of FePP compared to standard ferrous sulfate from 39 to 93% and from 75 to 125%, respectively, in rats. The authors suggested that the process of sterilization was responsible for this improvement, not the step of dissolution or dispersion of the Fe salt in the liquid formula (Theuer, 1985). Similar results were obtained by Wood et al. (1978) who showed that heat and pressure treatment resulted in an increased RBV of FePP, while the RBV of ferrous sulfate was unchanged in anemic chicks. On the contrary, Hurrell et al. (1991) reported that the RBV of FePP decreased from 75 to 21% in adult human subjects as a result of heat treatment (vacuum drying of a chocolate drink powder at 100°C). However, when the same labeled FePP was evaluated
several years later in an infant cereal, to which it was added after processing, RBV was only 15% indicating that also storage duration and/or conditions influence absorption of FePP (Hurrell et al., 2000). There is some evidence that processing increases bioavailability of added Fe when the process involves heating a predominantly aqueous food (wet-heat processing), whereas dry-heat processing has little effect on the bioavailability of added Fe (Lee, 1982).

Another study, evaluating the influence of physicochemical properties of powdered FePP on the Fe bioavailability in rats using the Hb repletion assay, suggested that the water content of the used FePP powder could be related to the Fe bioavailability and that the RBV was lower (72%) when the water content was relatively small compared to 82% when the water content was higher (Tsuchita et al., 1991). The authors also showed that the availability from powdered FePP was partly but not significantly improved from 66 to 79% by dispersing in skim milk and redrying which was possibly due to the formation of Fe-skim milk complexes in the preparation process resulting in improved bioavailability. However, the bioavailability of liquid FePP added to reconstituted milk and then dried showed a bioavailability similar to ferrous sulfate wherefore results lead to the recommendation that FePP should be used in an aqueous suspension form and treated with skim milk before drying when added to powdered infant formulas.

Summarizing the results from all the bioavailability studies, absorption values vary widely, depending on batch and processing indicating that the relative bioavailability of a Fe source should be used with caution, as it is only an approximate guide to potential absorption. Meal composition, the physicochemical environment provided by the food, and the enhancers and inhibitors of Fe absorption in a meal, together with the Fe status of the person consuming it, largely govern the level of Fe absorption (Hallberg, 1981; Clydesdale, 1983; Wortley et al., 2005).

Particle size reduction

Particle size distribution has been demonstrated to influence bioavailability of Fe sources that are not readily soluble in water or in gastric juice. As early as in 1973 Cook et al. (1973) demonstrated in a human study, that Fe absorption from H-reduced elemental Fe powder, with particle sizes between 5-10 μm, was equivalent
to ferrous sulfate. Two other studies showed that decreasing the particle size of elemental Fe powders by 50-60%, to a mean particle size of 7-10 μm, increases Fe absorption by ≈ 50% in rats (Motzok et al., 1975; Verma et al., 1977). Harrison et al. (1976) demonstrated that the relative bioavailability of ferric orthophosphate was highly correlated with acid solubility ($r = 0.99$) and negatively correlated with particle size ($r = 0.95$). RBV of 5 commercial samples of ferric orthophosphate with particle sizes in the range of < 1 μm to 15 μm was 6-46% in rats using the hemoglobin repletion test. In the same study, the RBV of a sample of electrolytic Fe which was separated into fractions of 7-10 μm and 27-40 μm was compared and showed a RBV for the finer fraction of 68-75% and of 27-29% for the coarser fraction. The difference in bioavailability between these two samples was confirmed by a collaborative study in rats and showed mean RBVs of 64 and 38% for the finer and coarser fraction, respectively (Fritz et al., 1974). In a recent rat study, the bioavailability of different elemental Fe powders was compared and carbonyl Fe, with a smaller particle size than the other tested compounds, had the highest RBV (Swain et al., 2003).

Several recent studies looked at the effect of particle size reduction of FePP on its bioavailability. In a human stable isotope study the RBV of FePP with an average particle size of 6.7 μm and 12.5 μm were compared in adult women consuming test meals of Fe fortified infant cereal and were found to be not significantly different with RBVs of 52 and 42%, respectively (Fidler et al., 2004a). However, using salt dual fortified with iodine and micronized FePP with an average particle size of 2.5 μm, demonstrated high bioavailability and efficacy in an intervention trial in Moroccan children (Zimmermann et al., 2003b). Another intervention trial using the same FePP compound encapsulated together with iodine and vitamin A and added to salt was efficacious in reducing ID and IDA prevalence (Zimmermann et al., 2004a). However, regular FePP would possibly have been as bioavailable as the micronized FePP in these two studies and in the second study, bioavailability of FePP might have been increased by the simultaneous addition of other micronutrients.

In recent years a proprietary super-dispersion technology has been identified producing emulsified SunActive Fe™, which has a low particle size (0.3 μm) and has been found to be as bioavailable as ferrous sulfate in humans (Berry, 2004). In a recent in vitro bioavailability trial whole-grain wheat chapatis fortified with SunActive Fe (the authors do not state whether they used the liquid or powder form) showed
significantly higher dialyzable Fe concentrations than those fortified with ferrous sulfate or regular FePP (Kloots et al., 2004). However, there was no significant difference between the Caco-2 cell absorption from ferrous sulfate, FePP or SunActive Fe probably due to the high standard deviations. Interestingly, the relative Fe dialyzability and Caco-2 cell absorption from chapatis made with emulsified Lipofer was not higher than that from chapatis made with ferrous sulfate or FePP most probably due to the higher particle size (7 μm) and possibly also due to the different composition compared to the SunActive Fe compound. Another recent study using an in vitro digestion/human colonic adenocarcinoma (CaCo-2) cell model showed that the bioavailability of SunActive Fe from fortified processed wheat cereal was 1 ½ times that of ferrous sulfate and more than 2 times that of ferrous fumarate (Wortley et al., 2005).

In a recent rat study the RBV of dispersible SunActive Fe™ was 104% that of ferrous sulfate compared to 56% for regular FePP (Sakaguchi et al., 2004). The first human stable isotope study with this compound has shown that Fe absorption from an infant cereal and a yogurt drink fortified with dispersible SunActive Fe™ was 83 and 94%, respectively, in adult women and was not significantly different to that from ferrous sulfate (Fidler et al., 2004c). However, it is unclear whether the high RBV of this FePP compound is due only to its small particle size or whether the surrounding emulsifiers may also play a role. In a recent stable isotope study with adult women, the absorption of SunActive Fe™ was tested in a wheat/milk infant cereal and in rice and compared to that of ferrous sulfate (D. Moretti, unpublished data, 2004). In the rice, SunActive Fe was either part of the extruded fortified rice grains or was added at the time of feeding to non fortified rice. Results of this study showed that RBVs are much lower than in the above mentioned study with values of 63% for the infant cereal and 15 and 25% for the unprocessed and processed rice, respectively. The absorption of 63% from the infant cereal in this study compared to the 83% absorption in an earlier study (Fidler et al., 2004c) could be due to a difference in particle size of the isotopically labeled SunActive Fe of 0.77 μm and 0.24 μm, respectively. However, the 63% absorption from the infant cereal is similar to the RBV of 54% from an infant cereal fortified with the same SunActive iron compound obtained in an earlier unpublished study (D. Moretti, unpublished data, 2002). The reasons for the very low bioavailability of SunActive Fe from the rice meal are not clear. There is some evidence that the RBV values of insoluble iron
compounds may vary with the food matrix used, as has previously been shown by Hallberg et al. (1986) with carbonyl iron. In addition, the higher particle size of 0.8 μm could also have decreased the bioavailability compared to the 0.3 μm of the commercial SunActive Fe product. Further studies with SunActive Fe are needed to answer the questions whether meal composition, particle size, emulsifiers or other factors influence the bioavailability of this compound.

Although, some of the studies indicate that particle size reduction to ≈ 0.3 μm may increase the RBV of FePP, the commercially available compound is prohibitively expensive and patented. The cost of the not patented micronized FePP with an average particle size of 2.5 μm, produced by conventional grinding, is ≈ 4 times the cost of ferrous sulfate on a per Fe basis (W. Vogl, personal communication, 2004). In general, processing costs to reduce particle size are high and could be a major barrier to its use in fortification programs in developing countries. However, if these issues can be resolved, small particle size FePP is a promising Fe fortificant due to its combination of inert sensory characteristics with good bioavailability. Still one major question remains: Is the bioavailability of micronized FePP better than the bioavailability of regular ferric pyrophosphate? As to date, no human studies exist in which the bioavailability of micronized ferric pyrophosphate is directly compared to that of regular pyrophosphate.

**Ferric pyrophosphate in food fortification**

FePP is preferred for the fortification of infant cereals and chocolate-drink powders because it does not cause objectionable changes in the acceptability of the food being fortified (Hurrell, 1985). It has been widely used to fortify infant cereals in Europe although it may sometimes provokes fat oxidation at higher levels of addition (> 10 mg Fe/100 g) (Hurrell, 1985). A study conducted by Hurrell et al. (1989a) performed organoleptic studies on stored material fortified with different Fe sources. Surprisingly, FePP showed similar negative effects such as rapid fat oxidation and off-odor as ferrous sulfate when added to a wheat cereal and to a wheat-milk cereal. But if FePP solubilized with either sodium citrate or ammonium citrate was added to a rice cereal and a mixed cereal there was only little evidence of fat oxidation and the odor scores were acceptable. Chocolate drink powders fortified with FePP and
reconstituted with cold, hot (80°C) and boiling water or milk were judged to be acceptable with respect to color and taste (Hurrell et al., 1991). In 1969, Sundaravalli et al. (1969) reported a simple, inexpensive method of preparing a shark liver oil emulsion containing FePP. The oil was quite stable at room temperature, did not develop any perceptible rancidity after storage for one year and the emulsion containing FePP was approximately 60% as effective as a comparable dose of ferrous sulfate in hemoglobin regeneration studies in anemic rats.

Due to the inert properties and the white color, FePP is a preferred Fe compound to fortify white food vehicles such as salt and rice. As early as in 1972 salt fortified with FePP was found to be suitable from a technical point of view, but not desirable from the point of view of Fe availability, showing negligible absorption (Narasinga Rao et al., 1972). The conclusion of the study was that if common salt is to be used as a vehicle for Fe fortification, better available fortificants should be sought. This resulted in no further investigation of FePP as Fe fortificant for salt until recently when FePP in a micronized form (average particle size $\approx 2.5 \, \mu m$) has been successfully used in two salt fortification trials in northern Morocco (Zimmermann et al., 2004a; 2004b). In the first trial micronized FePP was mixed into local salt together with KIO$_3$ (Zimmermann et al., 2004b). Storage tests showed no significant discoloration and no significant loss of iodine of the fortified salt compared to iodized salt after storage for 6 months and the dual fortified salt was highly efficacious in improving Fe status parameters and reducing IDA prevalence in school children. In the second trial, micronized FePP was encapsulated in palm oil together with KIO$_3$ and retinyl palmitate and the microcapsules were added to local salt (Zimmermann et al., 2004a). In this formulation as well, color was stable after 6 months of storage and the triple fortified salt was efficacious in improving Fe and vitamin A status in the study children. The combined fortification of salt with iodine, vitamin A and Fe may have improved the efficacy of each single micronutrient.

Rice is besides salt another difficult to fortify food vehicle. Fe can be added to rice by mixing the Fe compound into rice flour, adding water, extruding the mixture and this fortified extruded rice grains are then added to normal rice grains at a ratio of 1:100 or 1:200 (Moretti et al., 2005). In a study iron-fortified extruded rice grains were produced using different Fe compounds (Moretti et al., 2005). Acceptable rice grains, color- and texture-wise were produced by using regular FePP, micronized FePP
(average particle size ≈ 2.5 µm) or micronized dispersable SunActive Fe\textsuperscript{TM}. Sensory tests showed no difference between unfortified rice and rice fortified with one of the two micronized FePP compounds in both uncooked and cooked form. The efficacy of rice fortified with the 2.5 µm FePP compound fed as school lunch was tested in Indian school children and results look promising (D. Moretti, personal communication, 2005).

FePP was considered to be a compatible and non reactive Fe compound for the fortification of products such as milk or milk products (Tsuchita et al., 1991) but its water insolubility makes it difficult to be used to fortify liquid products. However, sodium citrate-solubilized FePP was reported to be satisfactory for fortifying whole milk or skim milk (Demott, 1971) but was found to give color problems in a later study (Hurrell, 1985). More recently, the development of SunActive Fe\textsuperscript{TM} offered new possibilities for Fe fortification of milk products, soft drinks, yogurt, yogurt drinks, ice cream, soups, and salad dressings since it disperses insoluble iron in liquid formulations without affecting the color and flavor of the product and, unlike regular FePP, it has equivalent bioavailability to ferrous sulfate (Sakaguchi et al., 2004). Product developers in Japan and Europe are using SunActive Fe to formulate Fe fortified milk, yogurt and sports drinks and in Korea it is used to fortify milks targeted to very small children (Smith, 2003). In addition to milk products, this FePP compound is potentially a suitable Fe fortificant for food vehicles that are difficult to fortify such as chocolate-drink powders, cereal products, bouillon cubes, iodized salt and rice.
Chapter 1

STUDY SITES

One part of the first and the second study presented in this thesis were carried out in Northern Morocco (Figure 1). The study site was a small rural village in a region of endemic goiter in the Rif Mountains (Zimmermann et al., 2000c). The village is about 800 m above sea level and has a temperate climate with an 8 months dry season (22-34°C, mean rainfall 23 cm/month) and a 4 months damp season (10-22°C, mean rainfall of 77 cm/month). It is a rural village isolated from commercial routes with most of the population producing their own food on small farms (Larbi, 1991). Although Morocco legislated mandatory salt iodization in 1997 it is estimated that only ≈ 45% of the population has access to iodized salt (N. Chaouki, personal communication, 2002) and the small local salt cooperative supplying the study village had not yet begun iodization during the study period due to financial constraints. Per capita salt and iron intakes in school-age children in this region are 7-12 g/d and 9-14 mg/d, respectively, with a Fe bioavailability estimated to be 1-4% from the local diet (Zimmermann et al., 2003b) when adjusted for low body iron stores (Cook et al., 1991). The prevalence of ID and IDA in this region was found to be 15 and 35%, respectively (Zimmermann et al., 2003b). Urinary iodine excretion was low with 17 μg/L and goiter prevalence was high with 71%. These results indicate that it is likely that many children suffer from both IDD and IDA.

Another part of the first study and the forth study were carried out in Côte d’Ivoire, West Africa (Figure 1). The study site for the first study was Abidjan, a large city on the tropical coast. The climate is tropical, with temperatures of ≥ 27°C and a relative humidity of ≥ 80% much of the year. During the ≈ 6 months rainy season, the village experiences daily drenching rains. Côte d’Ivoire legislated mandatory salt iodization in 1996. Abidjan is the entry point for edible salt imported from Senegal and Namibia and is further iodized if necessary in some of the salt factories in Abidjan. The forth study was conducted in the four primary schools in a rural village in the Dabou district of Côte d’Ivoire, 10 km from the southern coast. This village is about 50 km away from Abidjan with the same tropical climate. Most of the foods consumed are produced locally, and the staple food is cassava. Plantain, rice, yam and dried, smoked fish are eaten regularly. Daily iron intake of school children in the northern and central part of Côte d’Ivoire was estimated to be 3-13 mg with an estimated iron
bioavailability of 7-13% for individuals with a serum ferritin concentration of 20 µg/L (Staubli-Asobayire, 2000). Median daily salt intake in school children was 6.6 g in the north and 3.7 g in the center (Staubli-Asobayire, 2000).

ID and IDA prevalence in school children in two villages in western Côte d'Ivoire were found to be 50% and 27%, respectively (Zimmermann et al., 2000b). A study conducted in 4 regions of Côte d'Ivoire, including Abidjan and a village in southern Côte d'Ivoire showed a prevalence of ID in school children of 35-45% and no difference was found between the 4 regions (Staubli-Asobayire, 2000). IDA prevalence was highest in school children in Abidjan with 34% and lower in the rural village in southern Côte d'Ivoire with 23%. However, only 58% of the anemia was associated with iron deficiency indicating that as in most tropical developing countries anemia cases are often also due to other causes than iron deficiency such as other micronutrient deficiencies (Ramakrishnan, 2001), inflammatory disorders (Yip & Dallman, 1988), malaria (Fleming, 1981) or hemoglobinopathies (Shah, 2004).

Côte d'Ivoire is a malaria endemic country with a high prevalence of *Plasmodium falciparum* malaria as has been shown in several studies (Staubli Asobayire et al., 2001; Nzeyimana et al., 2002; Raso et al., 2004). Multiple infections are common and interactions complex as has recently been shown in a study in a rural village in Western Côte d'Ivoire with 500 participants covering all age groups (Raso et al., 2004). Three quarters of the population was infected with 3 or more parasites concurrently and the prevalence of *P. falciparum* (76%), hookworms (45%), *Entamoeba histolytica/E. dispar* (42%), and *Schistosoma mansoni* (40%) was high. Only 2% had no infection. This observation is in agreement with a earlier study in the same region, where poly parasitism was also a common feature among school children (Utzinger et al., 1999). Data from southern rural Côte d'Ivoire showed that 64% of school age children were infected with malaria parasites, 35% with hookworms, 8% with *Trichuris trichiura*, 25% with *Ascaris lumbricoides* and 28% with *S. mansoni* (Staubli-Asobayire, 2000).

In such areas other micronutrient deficiencies such as vitamin A, riboflavin, iodine and selenium deficiency are common. In southern rural Côte d'Ivoire 25% of school children had marginal vitamin A deficiency (Staubli-Asobayire, 2000). This is in agreement with another study conducted in western Côte d'Ivoire (Zimmermann et al., 2000b). Another study in the same region demonstrated a vitamin A deficiency prevalence of 45% in school children and 92% were selenium deficient (Hess, 2003).
which is in agreement with a study by Zimmermann et al. (2000d) in which all of the study children were selenium deficient. Only one study, also conducted in the western part of the country, looked at riboflavin deficiency (Arnaud et al., 2001). 74% of the studied subjects were riboflavin deficient but only 46% were selenium deficient. Before salt iodization started in 1996, the mountainous region of western Côte d'Ivoire was a region of endemic goiter (Latapie et al., 1981; Franke et al., 1999). A study conducted in school children in western Côte d'Ivoire, after the introduction of iodized salt, showed a median urinary iodine concentration of 162 μg/L but still a goiter prevalence of 59%, and 23% suffered from both, ID and goiter (Hess et al., 2002a). However, 4 years after salt iodization the goiter prevalence was lower in school children but was still 29% (Zimmermann et al., 2003a).

Although most data available are from the western part of Côte d'Ivoire, it can be estimated that the overall health situation is about the same in the southern part of the country having similar tropical climate. The study population was expected to be at high risk of parasitic infection, malaria and monotonous and poor quality diets (Staubli-Asobayire, 2000; Raso et al., 2004).

Figure 1: Map of Africa with the study sites in Morocco and Côte d'Ivoire
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Literature Review


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Chapter 1


Literature Review


Chapter 1


Dual Fortification of Salt with Iodine and Encapsulated Iron Compounds: Stability and Acceptability Testing in Morocco and Côte d’Ivoire

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Abstract

The stability of local salt dual-fortified with iodine and 19 different iron compounds (encapsulated compared to nonencapsulated forms of sulfate, fumarate, pyrophosphate, and elemental iron) was tested in Morocco and Côte d’Ivoire. Color and iodine content were measured after storage for 1, 2, 4 and 6 mo. Color acceptability was judged by standardized interviews. For most compounds, encapsulation did not protect against adverse sensory changes and iodine losses. However, 2 forms of ferric pyrophosphate, 1 small particle size (approximately 2.5 μm) and 1 micronized (approximately 0.5 μm), performed well and may be useful in salt fortification. Improvements in current encapsulation techniques are needed to allow encapsulated iron to be used in salt fortification.

Keywords: iodine, iron, fortification, encapsulation, salt
Introduction

Iodine-deficiency disorders (IDD) and iron-deficiency anemia (IDA) affect more than one third of the world’s population (WHO/UNICEF/UNU 1998; WHO/UNICEF/ICCIDD 2001). In West and North Africa, 20% to 30% of school children have both goiter and IDA (Zimmermann and others 2000b, 2000c), and IDA reduces the efficacy of iodine prophylaxis in areas of endemic goiter (Zimmermann and others 2000a, 2000b; Hess and others 2002). In many countries, iodization of salt is highly effective in reducing or eliminating IDD (WHO/UNICEF/ICCIDD 2001). However, there is no comparable, proven method for controlling IDA in populations on a national level (Hurrell 2002). Dual fortified salt (DFS) with iodine (I) and iron (Fe) could be an effective fortification strategy in regions with a high prevalence of IDD and IDA.

However, the fortification of salt with I and Fe is technically challenging (Madhavan Nair and others 1998; Venkatesh Mannar and Diosady 1998; Sattarzadeh and Zlotkin 1999; Sivakumar and others 2001). Water-soluble Fe compounds, which are the most bioavailable, tend to react with moisture and impurities in salt and produce unacceptable color changes (Venkatesh Mannar and Diosady 1998). Moreover, in the presence of ferrous ions and oxygen, I in salt is unstable because of catalytic oxidation of iodate or iodide to I$_2$, causing additional color changes and loss of I content. Insoluble Fe compounds, such as elemental Fe powders or Fe phosphate compounds, may cause fewer sensory changes but may be so poorly absorbed as to be of little nutritional value (Hurrell 2002). The ideal Fe compound for DFS would be one with high bioavailability that causes no sensory changes or I losses when added to salt. Narasinga Rao (1985) tested a variety of soluble and insoluble Fe compounds as potential salt fortificants. He concluded that none were satisfactory when added alone, and that the concurrent addition of either a stabilizer (such as sodium hexametaphosphate or orthophosphoric acid) or an absorption promoter (such as sodium acid sulfate) was required. However, placing a barrier around Fe by encapsulation could potentially reduce unwanted sensory changes and I losses in salt, while maintaining high bioavailability (Zimmermann and others 2003). Limited evidence from animal and human studies indicate that encapsulation with hydrogenated oils, methylcellulose, or maltodextrin have little or no effect on bioavailability. In rats, the bioavailability of several forms of encapsulated ferrous
sulfate was comparable to that of nonencapsulated ferrous sulfate (Hurrell and others 1989). In a recent intervention trial in northern Morocco, a form of dual-fortified salt containing ferrous sulfate hydrate encapsulated with partially hydrogenated soybean oil and potassium iodide was tested. Although this DFS was highly effective in reducing the prevalence of IDA and IDD in school children (Zimmermann and others 2003), it produced unwanted color changes in the salt during the damp winter season.

A variety of encapsulated Fe compounds, including sulfate, fumarate, pyrophosphate, and elemental Fe, are in development or commercially available worldwide. Most are encapsulated with hydrogenated oils and are used to fortify infant cereals and infant formulas, as well as other foodstuffs. To test their potential in salt, we dual-fortified local salt in temperate Morocco and tropical Côte d'Ivoire with I and different encapsulated Fe compounds. In a 6-mo trial, we compared their color, I stability and local acceptability.

**Materials and Methods**

**Study Sites**

The study site in Morocco was a small rural village in a region of endemic goiter in the Rif Mountains (Zimmermann and others 2000c). The village is about 800 m above sea level, the climate is temperate, and nearly all salt is supplied by a local cooperative. The salt is produced in drying ponds using water from a salty spring. Morocco legislated mandatory salt iodization in 1997, but due to financial constraints, this cooperative has not yet begun iodization. The fortified salts were prepared using unground, unwashed salt from this local cooperative. The study site in Côte d'Ivoire was Abidjan, a large city on the tropical coast. Côte d'Ivoire legislated mandatory salt iodization in 1996. Abidjan is the entry point for edible salt imported from Senegal, where it is produced in large quantities from salt lakes and widely distributed in West Africa. At the production site in Senegal, the salt is iodized at a low level by addition of potassium iodate. In Abidjan, the fortified salts were prepared using unwashed Senegalese salt obtained from a large importer.
Salt Fortification

To fortify the salts in Morocco, I was added as reagent-grade potassium iodide (4286, Sigma&Aldrich, Buchs, Switzerland) at a level of 40 μg/g salt (WHO/UNICEF/ICCIDD 2001). In Côte d’Ivoire, the native I content of the imported salt was determined to be about 17 μg/g by titration (UNICEF/PAMM/MI/ICCIDD/WHO 1995). Additional I was then added as reagent grade potassium iodate (60390, Fluka Chemie GmbH, Buchs, Switzerland) up to a level of 40 μg/g salt. First, a concentrated premix was made by adding either 830 mg KI or 620 mg KI03 to a 2-kg batch of salt using a small electric rotating drum mixer (RRM MINI 80, Engelsmann, Ludwigshafen, Germany) mixing at 26 rpm for 10 min. The Fe compound (described subsequently) was then added to the 2-kg premix and mixed for a further 10 min. The 2-kg concentrated premix containing both I and Fe was then mixed into a 14-kg batch of salt in a large electric rotating drum mixer (ELTE 650, Engelsmann, Ludwigshafen, Germany) at 30 rpm for 10 min in Morocco and in a planetary mixer (Hobart H400, London, UK) in Côte d’Ivoire. As standards for comparison, iodized salt also was prepared at both sites.

The Fe content, manufacturer, and capsule material of the 19 Fe compounds tested are shown in Table 1. We compared (1) electrolytic Fe to encapsulated electrolytic Fe, (2) ferric pyrophosphate to encapsulated ferric pyrophosphate, (3) ferrous fumarate to encapsulated ferrous fumarate (6 different capsules), and (4) ferrous sulfate and ferrous sulfate + 1% sodium hexametaphosphate (SHMP) to encapsulated ferrous sulfate (7 different capsule materials).

Particle size distribution of the encapsulated and nonencapsulated Fe compounds ranged from about 50 to 1000 µm, except for the 2 ferric pyrophosphate compounds, which had a mean particle size of 2.5 µm for the nonencapsulated compound and 0.5 µm for the encapsulated compound.

The salt fortification level chosen for the different Fe compounds was equivalent to 1 mg of Fe as ferrous sulfate per gram salt and was based on (1) the estimated relative bioavailability of the Fe compounds compared with ferrous sulfate (Hurrell 2002), (2) previous studies of daily salt intake in Morocco and Côte d’Ivoire (Hess and others 1999; Zimmermann and others 2003), and (3) a goal of supplying roughly the estimated daily iron requirement (FNBIM 2001). Therefore, the electrolytic iron and ferric pyrophosphate compounds were added to the salt at a level of 2 mg Fe per gram salt, and the ferrous sulfate and ferrous fumarate compounds were added at a
level of 1 mg Fe per gram salt. For the formula containing ferrous sulfate and SHMP, the SHMP (Chemische Fabrik Budenheim, Budenheim, Germany) was mixed into the salt together with the ferrous sulfate in the final mixing step described earlier.

**Stability testing**

All fortified salts as well as the native, non-fortified salt were divided into 10-kg and 5-kg portions in loosely woven high-density polyethylene (HDPE) bags. This type of bag, woven from large flat strands of HDPE, is used to package salt at both production sites. Two 300-g portions of all salts were stored in transparent low-density polyethylene (LDPE) bags typically used at the retail level and in markets. In both Morocco and Côte d’Ivoire, the bags were stored in clean, well-ventilated rooms out of direct sunlight, and evenly spaced on shelves to allow air circulation. The temperature and humidity at each site were measured daily and/or weekly. The mean temperature and relative humidity (SD) during 6 mo of storage were 19.1 °C (2.5) and 67.1 % (5.5) in Morocco, and 27.6 °C (1.3) and 80.3 % (4.1) in Côte d’Ivoire. At mixing and after storage for 1 and 2 mo, about 30-g aliquots of salt were collected in tightly sealed plastic containers, filled to minimize exposure to air, wrapped in aluminum foil to prevent exposure to light, and then frozen until analysis. Although there was no visible segregation during storage, to minimize potential inhomogeneity at sampling, each bag was manually mixed for 2 to 3 minutes before the aliquots were taken. For each salt, 3 aliquots were taken from the 10-kg bag, 2 aliquots from the 5-kg bag and 1 aliquot from each of the two 300-g bags. For those salts whose color was judged acceptable in local interviews after 4 mo of storage (discussed subsequently), additional aliquots were taken after 4 and 6 mo of storage.
<table>
<thead>
<tr>
<th>Iron compound</th>
<th>Capsule material/Additives</th>
<th>Iron (%)</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrolytic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Electrolytic iron</td>
<td>None</td>
<td>100</td>
<td>OMG Americas, Research Triangle Park, N.C., U.S.A.</td>
</tr>
<tr>
<td>2 Electrolytic iron (VITASHURE\textsuperscript{TM} 432)</td>
<td>Partially hydrogenated soybean oil</td>
<td>70</td>
<td>Balchem Corp., Slate Hill, N.Y., U.S.A.</td>
</tr>
<tr>
<td><strong>Pyrophosphate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Ferric pyrophosphate (3043448)</td>
<td>None</td>
<td>21</td>
<td>Dr. Paul Lohmann GmbH KG, 31857 Emmerthal, Germany</td>
</tr>
<tr>
<td>4 Ferric pyrophosphate (SUNACTIVE Fe-P80)</td>
<td>Dextrin, glycerol esters of fatty acids, sodium chloride, enzymatically hydrolyzed lecithin</td>
<td>8</td>
<td>Taiyo Kagaku Co., Ltd., Yokkaichi, Mie, Japan</td>
</tr>
<tr>
<td><strong>Fumarate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Ferrous fumarate (1942520)</td>
<td>None</td>
<td>33</td>
<td>Dr. Paul Lohmann</td>
</tr>
<tr>
<td>6 Ferrous fumarate (1942591)</td>
<td>Partially hydrogenated palm oil</td>
<td>16</td>
<td>Dr. Paul Lohmann</td>
</tr>
<tr>
<td>7 Ferrous fumarate (F080018)</td>
<td>Fully hydrogenated soybean oil</td>
<td>20</td>
<td>Watson Foods Co., Inc., West Haven, Conn., U.S.A.</td>
</tr>
<tr>
<td>8 Ferrous fumarate (P-4062)</td>
<td>Granulated with dextrin, cellulose, sodium hexametaphosphate, coated with approximately 30% soy stearine</td>
<td>28</td>
<td>Bio Dar Ltd., Yavne, Israel</td>
</tr>
<tr>
<td>9 Ferrous fumarate</td>
<td>Edible wax matrix (Micro release)</td>
<td>12</td>
<td>The Micronutrient Initiative, Ottawa, Ontario, Canada</td>
</tr>
<tr>
<td>10 Ferrous fumarate (9-3176)</td>
<td>Edible matrix of mono and diglycerides</td>
<td>18</td>
<td>Particle Dynamics, St. Louis, Mo., U.S.A.</td>
</tr>
<tr>
<td>11 Ferrous fumarate (9-4506)</td>
<td></td>
<td>21</td>
<td>Particle Dynamics</td>
</tr>
<tr>
<td><strong>Sulfate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Ferrous sulfate (dried) (3590540)</td>
<td>None</td>
<td>29</td>
<td>Dr. Paul Lohmann</td>
</tr>
<tr>
<td>13 Ferrous sulfate (heptahydrate) (3590460)</td>
<td>Sodium hexametaphosphate added during fortification</td>
<td>20</td>
<td>Dr. Paul Lohmann</td>
</tr>
<tr>
<td>14 Ferrous sulfate (monohydrate) (VITASHURE\textsuperscript{TM} 484)</td>
<td>Partially hydrogenated soybean oil</td>
<td>17</td>
<td>Balchem Corp.</td>
</tr>
<tr>
<td>15 Ferrous sulfate (monohydrate) (P-4065)</td>
<td>Mono and diglycerides of edible fatty acids</td>
<td>15</td>
<td>Bio Dar Ltd.</td>
</tr>
<tr>
<td>16 Ferrous sulfate (monohydrate) (P-4061)</td>
<td>Cellulose derivative</td>
<td>27</td>
<td>Bio Dar Ltd.</td>
</tr>
<tr>
<td>17 Ferrous sulfate (dried) (3590593)</td>
<td>Partially hydrogenated palm oil</td>
<td>23</td>
<td>Dr. Paul Lohmann</td>
</tr>
<tr>
<td>18 Ferrous sulfate (dried) (F080007)</td>
<td>Fully hydrogenated soybean oil</td>
<td>20</td>
<td>Watson Foods Co., Inc.</td>
</tr>
<tr>
<td>19 Ferrous sulfate (heptahydrate)</td>
<td>Soy stearine</td>
<td>20</td>
<td>The Micronutrient Initiative</td>
</tr>
<tr>
<td>20 Ferrous sulfate (dried) (9-4354)</td>
<td>Stearic acid</td>
<td>20</td>
<td>Particle Dynamics</td>
</tr>
</tbody>
</table>
Acceptability testing
Color acceptability of the fortified salts was determined after 4 mo of storage in both Morocco and Côte d'Ivoire. At the local market, a native interviewer showed local adults (n=82 in Morocco; n=50 in Côte d'Ivoire) unmarked 100-g iodized salt and dual-fortified salt samples side-by-side in identical clear polyethylene bags on white backgrounds. The subjects were asked to judge the color of the salts by assigning them numbers: 1 = I would never buy this salt; 2 = I would possibly buy this salt; 3 = I would certainly buy this salt. A salt was judged acceptable if its average score from the interview was ≥2.

Laboratory Analysis
The mineral composition of the native salts from Morocco and Abidjan was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Modula, Spectro Analytical Instruments GmbH & Co. KG, Kleve, Germany) and the particle size distribution measured by sieve analysis using sieves with the following mesh size: 0.125, 0.250, 0.500, 0.800, 1.000, 1.400, 2.000, 2.800, and 4.000 mm and a shaking time of 10 minutes.

Color stability. At baseline, 1, 2, 4 and 6 mo, the color of the fortified salts was compared with that of native, nonfortified salt by panel visual inspection in indirect natural light of unmarked 20-g samples side-by-side on white backgrounds. Color was also determined at each time point by colorimetry. Approximately 10 g of salt was transferred into a round glass container for color determination on the Hunter scale by a Spectral Photometer (Chroma-Meter CR-310, Minolta [Schweiz] AG, Dietikon, Switzerland), using illuminant D65 (average daylight, including ultraviolet spectra), a 0° observer angle, with a large reflectance spectrum. On the Hunter scale, the L value is a measure of light reflection. An L value of 100 is pure white; an L value of zero is pure black. The a value is a measure of redness (positive a values) and greenness (negative a values). The b value is a measure of yellowness (positive b values) and blueness (negative b values). Each sample was measured 3 times and the glass container rotated 90° after each measurement. The color of the dual-fortified salts was then compared with that of iodized salt. The color difference was expressed by a single value $\Delta E_{ab}$, which contained the absolute value of the color differences.
Stability of dual fortified salt

difference but not the direction of the difference. \( \Delta E_{ab} \) was calculated using the following equation:

\[
\Delta E_{ab} = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}
\]

where \( \Delta L \), \( \Delta a \), and \( \Delta b \) describe the difference between the color of the sample (dual-fortified salt) and the reference color (iodized salt).

**Iodine stability.** The high concentration of Fe in dual-fortified salt interferes with the reaction of sodium thiosulfate and free I (Werlen 2000) and thereby precludes accurate determination of I content by titration with sodium thiosulfate (UNICEF/PAMM/MI/ICCIDD/WHO 1995). Therefore, salt-I concentration was measured using a modification of the Sandell-Kolthoff reaction originally described for the determination of urinary I (Pino and others 1996). Ten-gram salt aliquots were diluted to 100 g with deionized, filtered water and placed into an ultrasound bath (Branson 5210, Branson Ultrasonic Corp., Danbury, Conn., U.S.A.) for 40 min to dissolve the salt. Eight grams of the dissolved salt sample were taken and again diluted to 100 g. From this dilution an aliquot of 250 µl was taken and the I content measured using the method of Pino and others (1996). This method for I determination in dual-fortified salt was compared to isotope abundance ratio measurements by inductively coupled plasma-mass spectrometry (ICP-MS) and agreement between the 2 methods was excellent \( (r^2=0.97) \) (data not shown). The pooled coefficient of variation (CV_p) using the modified Sandell-Kolthoff method at 5 and 30 µg I per gram salt was 9% and 6%, respectively, in the Ivorian salt and 22% and 7% in the Moroccan salt.

**Iron content.** The fortification level of Fe in the baseline samples was verified by atomic absorption spectroscopy. An aliquot of salt (0.25 to 2 g, depending on the amount of fortified Fe in the salt) was added to 1 mL concentrated HCl and 10 mL distilled, deionized water and the solution heated to boiling. After cooling to room temperature, samples were diluted with water to 25 g. Fe content was then analyzed by the standard addition method using flame atomic absorption spectroscopy (SpectrAA-400, Varian, Mulgrave, Australia).
Statistical Analysis

Colorimetry and I data were expressed as mean (SE) for each dual-fortified salt at 0, 1, 2, 4, and 6 mo. Pearson correlations were done between the mean acceptability score for a salt from interviews and its color difference ($\Delta E_{ab}$ value). I content of salts analyzed at 4 and 6 mo were compared using independent-samples t tests. P values <0.05 were considered significant.

Results and Discussion

The water content, selected mineral composition and particle size of the native salts from Morocco and Côte d'Ivoire are shown in Table 2.

Table 2 - Native composition and particle size distribution of salts from Morocco and Côte d'Ivoire

<table>
<thead>
<tr>
<th>Component</th>
<th>Moroccan salt (%)</th>
<th>Ivorian salt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>95.4</td>
<td>93.3</td>
</tr>
<tr>
<td>Water</td>
<td>0.18</td>
<td>1.55</td>
</tr>
<tr>
<td>Calcium sulfate</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>&lt; 0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Iron</td>
<td>1.76 mg/kg</td>
<td>12.1 mg/kg</td>
</tr>
<tr>
<td>Potassium</td>
<td>78.4 mg/kg</td>
<td>106 mg/kg</td>
</tr>
<tr>
<td>Insolubles</td>
<td>0.14</td>
<td>0.83</td>
</tr>
<tr>
<td>Particle size distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q50, (mm)</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Q90, (mm)</td>
<td>3.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Color stability

To approximate overall stability from the production site through retail to the household, the results for color differences from the HDPE and LDPE bags were averaged. Color differences comparing the dual-fortified salts with the iodized salts (combined data from both the large and small bags) are shown in Table 3 and 4 for the Moroccan and Côte d'Ivoire salts, respectively. There was a rapid and marked color change in many of the dual-fortified salts during mixing, particularly the ones fortified with elemental Fe, which turned dark brown almost immediately. In general, the color change in the Moroccan salts was greater than in the Ivorian salts (Table 3
and 4). This was particularly evident in the salts fortified with ferrous sulfate, which turned dark yellow-orange in Morocco but only slightly yellow in Côte d'Ivoire. There was no consistent pattern as to which color value (L, a, or b) was most affected by the different DFS.

The calculated color difference was a good predictor of local acceptability of the salt color as judged by the interviews. In both countries, interviewees rated as acceptable those salts with a color difference (ΔE<sub>ab</sub> value) less than 10. There was a good correlation between the mean acceptability score for a salt from the interviews and its color difference (ΔE<sub>ab</sub> value) in Morocco and Côte d'Ivoire (r<sup>2</sup>= 0.76 and 0.49, respectively). In Morocco, the color of 4 of the dual-fortified salts (nr 3, nonencapsulated ferric pyrophosphate; nr 9, encapsulated ferrous fumarate with SHMP; nr 10, encapsulated ferrous fumarate in edible wax; nr 13, ferrous sulfate with SHMP) were judged acceptable. In Côte d'Ivoire, the same 4 compounds were judged acceptable along with nr 4 (encapsulated ferric pyrophosphate, not tested in Morocco because of insufficient availability of product).
Table 3 - Mean color difference (SE) of dual-fortified salts compared with iodized salt determined by colorimetry and acceptability of dual-fortified salts in Morocco

<table>
<thead>
<tr>
<th>Sample</th>
<th>Months of storage</th>
<th>Color change after 2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>26.9 (0.2)</td>
<td>28.6 (0.2)</td>
</tr>
<tr>
<td>2</td>
<td>14.2 (0.2)</td>
<td>19.5 (0.2)</td>
</tr>
<tr>
<td>3</td>
<td>6.7 (0.1)</td>
<td>8.4 (0.1)</td>
</tr>
<tr>
<td>5</td>
<td>23.6 (0.1)</td>
<td>27.2 (0.2)</td>
</tr>
<tr>
<td>6</td>
<td>9.4 (0.1)</td>
<td>9.4 (0.1)</td>
</tr>
<tr>
<td>7</td>
<td>10.1 (0.2)</td>
<td>14.4 (0.8)</td>
</tr>
<tr>
<td>8</td>
<td>11.5 (0.1)</td>
<td>12.5 (0.6)</td>
</tr>
<tr>
<td>9</td>
<td>2.8 (0.2)</td>
<td>2.7 (0.2)</td>
</tr>
<tr>
<td>10</td>
<td>7.0 (0.2)</td>
<td>6.6 (0.4)</td>
</tr>
<tr>
<td>11</td>
<td>14.0 (0.2)</td>
<td>19.0 (0.4)</td>
</tr>
<tr>
<td>12</td>
<td>24.9 (0.1)</td>
<td>27.1 (0.5)</td>
</tr>
<tr>
<td>13</td>
<td>12.5 (0.2)</td>
<td>9.4 (0.3)</td>
</tr>
<tr>
<td>14</td>
<td>26.1 (0.1)</td>
<td>27.8 (0.2)</td>
</tr>
<tr>
<td>15</td>
<td>26.1 (0.1)</td>
<td>26.6 (0.4)</td>
</tr>
<tr>
<td>16</td>
<td>25.9 (0.1)</td>
<td>26.9 (0.4)</td>
</tr>
<tr>
<td>17</td>
<td>24.1 (0.1)</td>
<td>25.8 (0.5)</td>
</tr>
<tr>
<td>18</td>
<td>23.7 (0.1)</td>
<td>26.3 (0.3)</td>
</tr>
<tr>
<td>19</td>
<td>25.0 (0.1)</td>
<td>27.0 (0.1)</td>
</tr>
<tr>
<td>20</td>
<td>21.8 (0.1)</td>
<td>24.5 (0.2)</td>
</tr>
</tbody>
</table>

*aClosest to zero = color closest to iodized salt
*b1, electrolytic Fe; 2, encapsulated (EC) electrolytic Fe; 3, ferric pyrophosphate; 5, ferrous fumarate; 6 to 11, EC ferrous fumarate; 12, ferrous sulfate; 13, ferrous sulfate + SHMP; 14 to 20, EC ferrous sulfate
## Table 4 - Mean color difference (SE) of dual-fortified salts compared with iodized salt determined by colorimetry and acceptability of dual-fortified salts in Côte d'Ivoire

<table>
<thead>
<tr>
<th>Sample</th>
<th>Months of storage</th>
<th>Color change after 2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>27.0 (0.1)</td>
<td>30.1 (0.3)</td>
</tr>
<tr>
<td>2</td>
<td>13.2 (0.1)</td>
<td>23.0 (0.8)</td>
</tr>
<tr>
<td>3</td>
<td>3.6 (0.1)</td>
<td>3.3 (0.2)</td>
</tr>
<tr>
<td>4</td>
<td>1.7 (0.1)</td>
<td>3.1 (0.6)</td>
</tr>
<tr>
<td>5</td>
<td>13.4 (0.1)</td>
<td>16.2 (0.5)</td>
</tr>
<tr>
<td>6</td>
<td>6.3 (0.1)</td>
<td>9.7 (0.5)</td>
</tr>
<tr>
<td>7</td>
<td>6.1 (0.1)</td>
<td>12.0 (0.4)</td>
</tr>
<tr>
<td>8</td>
<td>4.9 (0.1)</td>
<td>11.7 (0.5)</td>
</tr>
<tr>
<td>9</td>
<td>1.8 (0.1)</td>
<td>1.9 (0.4)</td>
</tr>
<tr>
<td>10</td>
<td>4.2 (0.1)</td>
<td>6.3 (0.7)</td>
</tr>
<tr>
<td>11</td>
<td>6.1 (0.1)</td>
<td>10.5 (0.6)</td>
</tr>
<tr>
<td>12</td>
<td>6.1 (0.0)</td>
<td>8.9 (0.6)</td>
</tr>
<tr>
<td>13</td>
<td>1.7 (0.1)</td>
<td>5.0 (0.2)</td>
</tr>
<tr>
<td>14</td>
<td>9.1 (0.1)</td>
<td>8.2 (0.5)</td>
</tr>
<tr>
<td>15</td>
<td>6.1 (0.1)</td>
<td>12.2 (0.7)</td>
</tr>
<tr>
<td>16</td>
<td>9.0 (0.1)</td>
<td>11.8 (1.0)</td>
</tr>
<tr>
<td>17</td>
<td>7.0 (0.0)</td>
<td>10.0 (0.4)</td>
</tr>
<tr>
<td>18</td>
<td>6.0 (0.1)</td>
<td>9.6 (1.0)</td>
</tr>
<tr>
<td>19</td>
<td>9.7 (0.1)</td>
<td>11.4 (0.5)</td>
</tr>
<tr>
<td>20</td>
<td>5.7 (0.1)</td>
<td>11.1 (0.5)</td>
</tr>
</tbody>
</table>

- Closest to zero = color closest to iodized salt
- 1, electrolytic Fe; 2, encapsulated (EC) electrolytic Fe; 3, ferric pyrophosphate; 4, EC ferric pyrophosphate; 5, ferrous fumarate; 6 to 11, EC ferrous fumarate; 12, ferrous sulfate; 13, ferrous sulfate + SHMP; 14 to 20, EC ferrous sulfate
Iodine stability

To approximate overall I stability from the production site through retail to the household, the results for I stability from the HDPE and LDPE bags were averaged. Changes in I concentration in the dual-fortified salts and iodized salts containing no added Fe (combined data from both the large and small bags) are shown in Table 5 and 6 for Morocco and Côte d'Ivoire, respectively. In Morocco, the I concentration in the dual-fortified salts containing elemental Fe, ferric pyrophosphate, and ferrous fumarate was stable, with all compounds retaining > 90% of their original I content at 2 mo, except nr 3 (ferric pyrophosphate). The I stability of the encapsulated and nonencapsulated elemental Fe and fumarates was comparable. The two encapsulated fumarates whose color was judged acceptable (nr 9, nr 10) still had > 95% of their initial I content at 6 mo with no significant iodine loss compared with losses in iodized salt. In contrast, the I stability in the salts containing ferrous sulfate varied greatly. Three of the sulfates (nr 13, nr 14, nr 19) retained > 75% of their original I content at 2 mo, whereas the remaining sulfates retained < 25% at 2 mo. Iodine content of the other 2 salts with acceptable color (nr 3, nr 13) were significantly lower than iodized salt (p<0.01) at 6 mo.

The I stability of the fortified salts in Côte d'Ivoire was sharply lower than in Morocco. After 2 mo of storage, all of the salts, except for the two forms of pyrophosphate (nr 3, nr 4) and 1 encapsulated fumarate (nr 10), had lost > 50% of their original I content. These 3 salts, measured again at 6 mo, retained only about 30% of their I content. Similar to the pattern in the Moroccan salt, the salts containing ferrous sulfate generally performed poorly compared to the other Fe compounds, and the I stability of the encapsulated and nonencapsulated Fe compounds was comparable. Even the iodized salt (KIO₃) in Côte d'Ivoire retained only 41% of its original I content at 2 mo, in contrast to the excellent stability of the I (as KI) in the Moroccan iodized salt. There were no significant differences in the iodine content of the 5 salts analyzed at 4 and 6 mo compared with iodized salt. There was no correlation between color difference (ΔEab value) and iodine stability in the salts in Morocco and Côte d'Ivoire.

In Morocco, there was no significant difference in I stability when the small LDPE and the large woven HDPE bags were compared. After 2 mo of storage, the mean I
retention (SE) in the small LDPE bags and the large HDPE bags was 66.1% (6.3) and 66.1% (3.8), respectively. In contrast, in Côte d'Ivoire, I stability in the large HDPE bags was significantly reduced compared with the small LDPE bags (p<0.001). After 2 mo of storage, the mean I retention (SE) in the LDPE bags was 49.4% (7.1) compared with only 19.9% (2.3) in the woven HDPE bags.

Table 5 - Mean iodine content (SE) of dual-fortified salts in Morocco expressed as a percentage of the initial content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Months of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
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<tr>
<td>1</td>
<td>81.5 (8.1)</td>
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<td>2</td>
<td>91.0 (7.0)</td>
</tr>
<tr>
<td>3</td>
<td>78.6 (9.4)</td>
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<tr>
<td>5</td>
<td>89.2 (4.7)</td>
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<tr>
<td>6</td>
<td>91.8 (3.9)</td>
</tr>
<tr>
<td>7</td>
<td>99.2 (5.5)</td>
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<tr>
<td>8</td>
<td>94.9 (4.0)</td>
</tr>
<tr>
<td>9</td>
<td>93.4 (5.5)</td>
</tr>
<tr>
<td>10</td>
<td>96.1 (4.3)</td>
</tr>
<tr>
<td>11</td>
<td>83.1 (3.2)</td>
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<tr>
<td>12</td>
<td>16.8 (2.5)</td>
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<td>13</td>
<td>80.6 (4.5)</td>
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<td>14</td>
<td>86.3 (3.9)</td>
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<td>16</td>
<td>46.1 (2.2)</td>
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<td>17</td>
<td>28.0 (1.5)</td>
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<td>18</td>
<td>50.2 (3.6)</td>
</tr>
<tr>
<td>19</td>
<td>94.3 (2.9)</td>
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<tr>
<td>20</td>
<td>16.5 (2.0)</td>
</tr>
<tr>
<td>iodized</td>
<td>83.6 (2.9)</td>
</tr>
</tbody>
</table>

*1, electrolytic Fe; 2, encapsulated (EC) electrolytic Fe; 3, ferric pyrophosphate; 5, ferrous fumarate; 6 to 11, EC ferrous fumarate; 12, ferrous sulfate; 13, ferrous sulfate + SHMP; 14 to 20, EC ferrous sulfate
Table 6 - Mean iodine content (SE) of dual-fortified salts in Côte d’Ivoire expressed as a percentage of the initial content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Months of storage</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
<tr>
<td>1</td>
<td>54.7 (10.0)</td>
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<td>2</td>
<td>60.8 (24.2)</td>
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<td>3</td>
<td>66.4 (5.9)</td>
</tr>
<tr>
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<td>75.0 (5.2)</td>
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<tr>
<td>5</td>
<td>55.1 (14.9)</td>
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<td>29.1 (6.5)</td>
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<tr>
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<td>33.3 (11.0)</td>
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<td>43.2 (13.1)</td>
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<td>39.2 (18.1)</td>
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<td>70.9 (14.9)</td>
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<tr>
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<td>19.7 (4.7)</td>
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<tr>
<td>12</td>
<td>4.7 (1.7)</td>
</tr>
<tr>
<td>13</td>
<td>83.0 (23.3)</td>
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<tr>
<td>14</td>
<td>30.2 (7.7)</td>
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<tr>
<td>15</td>
<td>8.9 (4.3)</td>
</tr>
<tr>
<td>16</td>
<td>21.7 (7.9)</td>
</tr>
<tr>
<td>17</td>
<td>9.1 (2.7)</td>
</tr>
<tr>
<td>18</td>
<td>21.0 (5.7)</td>
</tr>
<tr>
<td>19</td>
<td>44.0 (12.7)</td>
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<td>20</td>
<td>3.2 (1.9)</td>
</tr>
<tr>
<td>iodized</td>
<td>72.1 (19.8)</td>
</tr>
</tbody>
</table>

\*1, electrolytic Fe; 2, encapsulated (EC) electrolytic Fe; 3, eerric pyrophosphate; 4, EC ferric pyrophosphate; 5, eerrous fumarate; 6 to 11, EC eerrous fumarate; 12, ferrous sulfate; 13, ferrous sulfate + SHMP; 14 to 20, EC ferrous sulfate

**Implications**

Most of the dual fortified salts developed yellow or brown off-colors during storage. The color changes were likely the result of two reactions: (1) the formation of ferric oxides of Fe with a rusty orange-brown color; and (2) the formation of I₂, which has an intense dark brown color. For 15 of the 19 dual-fortified salts, including all of the elemental Fe and ferrous sulfates (whether encapsulated or not), color change was judged unacceptable after 4 mo of storage in both Morocco and Côte d’Ivoire. The 1 sulfate that maintained acceptable color was the formula originally developed by the Indian Natl. Inst. of Nutrition (Narasinga Rao 1994) containing ferrous sulfate, KI, and 1% SHMP as an Fe-chelating agent (nr 13). This formula did not show significant color change in the Ivorian salt and remained white with small yellow spots in the Moroccan salt, even after 6 mo of storage. The reduction in color changes when SHMP is added...
as a stabilizing agent to dual-fortified salt has been previously demonstrated (Narasinga Rao 1994). Because of its affinity for moisture, in tropical climates with high humidity such as Côte d’Ivoire, SHMP reduces oxidation of ferrous sulfate (Ranganathan and others 1996). Diosady and others (2002) observed a pale yellow color in dual-fortified salt containing ferrous sulfate, KI, and SHMP, and suggested the color change was due to oxidation of the ferrous Fe. The color reverted to white after storage at high temperature and humidity, and the authors attributed this to the formation of white ferric phosphate.

Two of the salts fortified with encapsulated ferrous fumarate were judged acceptable at 4 mo. Because ferrous fumarate is poorly water soluble, reactions with I and salt impurities may be less rapid than with ferrous sulfate. These salts appeared slightly gray with small dark flecks of encapsulated ferrous fumarate, which had a dark red color even when encapsulated. Diosady and others (2002) observed rapid discoloration of salt dual-fortified with ferrous fumarate and iodate, which was attributed to the oxidation of fumarate by iodate to form dark brown ferric fumarate. They found that salt containing ferrous fumarate and iodide showed more acceptable color, flavor, and odor. This is in contrast to our results, in which nearly all of the ferrous fumarate fortified salts (even when encapsulated) turned unacceptably brown in salts fortified with either KIO₃ or KI. One of the acceptable salts, nr 9, was fortified with encapsulated ferrous fumarate in soy stearine and granulated with dextrin, cellulose, and SHMP. Its color stability was likely to be at least partly due to the presence of SHMP. Salt nr 10, fortified with encapsulated ferrous fumarate in an edible wax matrix performed well and showed only a slight color difference compared with baseline color. The dual-fortified salts in Morocco and Côte d’Ivoire containing encapsulated and nonencapsulated ferric pyrophosphate, both the micronized (approximately 0.5 μm) and small particle size (approximately 2.5 μm) forms, showed only a slight beige discoloration. Their good color stability was likely due to the fact that Fe phosphate compounds are insoluble in water and only poorly soluble in dilute acid, which reduces their reactivity, even in salt with a high moisture content.

Despite using a two step process to thoroughly mix the iodine into the salts, the large variability in the iodine content found during storage in several of the salts was likely due to inhomogeneity of the I content. Several factors probably contributed to our
findings that I retention in the Moroccan salt was generally higher than in the Ivorian salt. First, there is a considerable difference in the climate of these 2 regions. Whereas northern Morocco is dry and temperate, coastal Côte d'Ivoire has a tropical climate with high temperatures and humidity. In the Ivorian salt, moisture from the air readily penetrated through the woven HDPE bags, causing the salt to become palpably damp. The high moisture content probably accelerated I losses. Similar observations were made by Diosady and others (2002), who tested Fe and I compounds in salt under varying environmental conditions and found accelerated I losses during storage at higher temperatures (40°C) and humidity (100%). The Ivorian salt also has higher amounts of calcium and magnesium sulfates which, because of their hygroscopic nature, may also accelerate I losses from salt. Ranganathan and others (1996) reported that I loss in fortified salt was proportional to moisture and magnesium contents of the salt.

Another factor which may explain the varying patterns of iodine loss from salt in the 2 regions is that different I compounds were used for fortification. The salt imported from Senegal to Côte d'Ivoire is already lightly iodized with KIO₃, so we fortified this salt with KIO₃ instead of KI, the compound used in Morocco. In salt fortified with KIO₃, ferrous compounds may be oxidized by iodate producing I₂, which easily sublimes and is lost. This reaction occurs more rapidly in salt fortified with ferrous sulfate because of its high solubility compared with the other Fe compounds (Hurrell 1985). Therefore, Ivorian salts fortified with ferrous sulfate lost I rapidly (except for the formula containing SHMP). Ferrous fumarate-fortified salts lost I as well, but at a slower rate than ferrous sulfate-fortified salts. Similar results were reported by Diosady and others (2002), who compared dual-fortified salts containing ferrous sulfate or ferrous fumarate together with KIO₃ and found that salts containing ferrous fumarate retained more I than those containing ferrous sulfate. I content was most stable in Ivorian salts fortified with ferric pyrophosphate, which is consistent with the inability of ferric Fe to reduce iodate to I₂.

In Morocco, salts fortified with KI and ferrous sulfate showed more rapid I losses compared with other Fe compounds. I content of salts fortified with ferrous fumarate remained stable or showed only minor losses, probably because of their reduced solubility in water compared with sulfate and the dry storage conditions. In contrast to its superior performance in the Ivorian salt, in Morocco the ferric pyrophosphate-fortified
Stability of dual fortified salt

Salt lost substantial I, probably because of the interaction of Fe$^{3+}$ and KI resulting in the formation and sublimation of I$_2$.

I loss from iodized salt without added Fe was much more rapid in Côte d'Ivoire than in Morocco. The I content in the Moroccan iodized salt remained stable after 6 mo of storage. In contrast, the I retention of iodized salt stored in woven HDPE bags in Côte d'Ivoire was < 10% after 6 mo of storage, whereas retention was about 80% when stored in LDPE bags. The influence of humidity and packaging materials on the stability of I in salt from tropical and subtropical climates was shown by Diosady and others (1997), who demonstrated that high humidity resulted in rapid loss of I from woven HDPE bags, whereas LDPE packaging largely prevented I losses. One alternative to minimize I losses in dual-fortified salt in Côte d'Ivoire could be the simultaneous encapsulation of I to prevent its interactions with Fe and salt impurities. Diosady and Venkatesh Mannar (2000) have reported that a dual-fortified salt containing dextrin-encapsulated KI showed minimal I losses after 12 mo at 40°C and 100% RH.

**Conclusions**

In general, Fe encapsulation did not greatly reduce either color change or I losses from dual-fortified salts. This suggests that currently available encapsulation techniques for Fe do not sufficiently reduce moisture penetration and/or Fe solubility when the encapsulated Fe is mixed into salt. Mechanical abrasion of the capsules during mixing also may contribute by damaging capsules and exposing and/or freeing Fe. Further refinements in capsule design will be needed to allow encapsulated Fe to be used as a salt fortificant. Because of moisture in the salt, elemental Fe compounds oxidized and rapidly turned the salt dark brown after mixing in both countries, and would appear to be of little value. Generally, salts fortified with ferrous sulfate lost I rapidly in both countries and turned unacceptably yellow. Because of the high solubility of ferrous sulfate, it is likely that only by adding a stabilizing agent like SHMP, or possibly a reducing agent such as ascorbic acid, could color changes and I losses be reduced. Ferrous fumarate-fortified salts were relatively more stable to I losses, but were associated with unacceptable reddish-brown color changes. One fumarate, encapsulated in an edible wax matrix, had acceptable color and did not appear to accelerate I losses from the salt, even in Côte d'Ivoire. Although promising, the
bioavailability of iron encapsulated in edible wax has not been tested. Overall, the best-performing dual-fortified salt in both regions were those containing ferric pyrophosphate. Although ferric pyrophosphate with a particle size ≥ 10 µm has a relative bioavailability of only 20% to 70% compared with ferrous sulfate, reducing its particle size to about 0.5 µm increases its bioavailability in humans (Fidler and others 2003). After 4 mo of storage, the color of the salts fortified with ferric pyrophosphate was acceptable, and they retained 90% and 56% of their original I content in Morocco and Côte d'Ivoire, respectively. Packaging material had a significant influence on I stability in tropical Côte d'Ivoire, where I losses from woven HDPE bags was greater than from LDPE bags. Although Fe encapsulation may improve stability of dual-fortified salts, process costs for encapsulation are high and unless reduced, may limit application in developing countries. Current cost estimates for encapsulated ferrous sulfate in partially hydrogenated soybean oil are 3 to 4 times the cost of nonencapsulated ferrous sulfate (Zimmermann and others, 2003), but the price of the former could drop substantially if production were to expand to a larger scale.

Acknowledgments

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Stability of dual fortified salt

References


Development, Stability and Sensory Testing of Microcapsules Containing Iron, Iodine, and Vitamin A for use in Food Fortification


Human Nutrition Laboratory and *Laboratory of Food Process Engineering, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, Zürich, Switzerland.

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Abstract

Iodine, vitamin A and iron deficiencies are important public health problems in developing countries and often coexist in vulnerable groups, such as pregnant women and young children. Food fortification can be a sustainable, cost-effective strategy to combat these deficiencies. In remote, rural areas of subsistence farming, salt may be one of few regularly purchased food items, and is therefore likely to be a good food vehicle for fortification. However, fortification of salt is challenging due to the white color and highly reactive impurities, and added micronutrients often cause color changes. Encapsulation may prevent or reduce these reactions. Potassium iodate, retinyl palmitate and ferric pyrophosphate were microencapsulated in hydrogenated palm fat by spray cooling. The size and morphology of the sprayed microparticles were analyzed, and losses of iodine and vitamin A during spraying were determined. The microcapsules were added to local salt in Morocco. During storage for 6 mo, color change in the triple fortified salt (TFS) was acceptable and iodine losses of ≈20% comparable to the iodized salt (IS). Stability of retinyl palmitate was excellent, resulting in losses of only ≈12% after 6 mo of storage. Sensory tests were performed with typical Moroccan dishes cooked with either TFS or IS by triangle testing. No sensory difference was detectable and overall acceptability of the salt was good. Encapsulation by spray cooling produces highly stable microcapsules containing iodine, vitamin A, and iron for salt fortification in Africa. Such capsules may also be used to fortify other dry matrices (for example, sugar, flour).

Key words: fortification, salt, micronutrients, microencapsulation, spray cooling
Introduction

Iodine, vitamin A and iron deficiencies are important in global public health problems, particularly for preschool children and pregnant women in low-income countries (Nutrition for Health and Development (NHD) /Sustainable Development and Healthy Environments (SDE)/World Health Organization (WHO) 2000). These deficiencies are mainly due to a monotonous, poor-quality diets that do not meet nutrient requirements (Tontisirin and others 2002). In countries, where existing food supplies and/or limited access fail to provide adequate levels of these nutrients in the diet, food fortification is a promising approach. In many countries, iodization of salt is highly effective in reducing or eliminating iodine deficiency disorders (IDD) (WHO/UNICEF/ICCIDD 2001). In Central America, sugar has been successfully fortified with vitamin A (Dary and Mora 2002). However, there is no comparable, proven method for controlling iron deficiency anemia (IDA) in populations on a national level (Hurrell 2002b). In addition, studies in animals and humans have shown that IDA impairs thyroid metabolism (Hess and others 2002a; Hess and others 2002b; Zimmermann and others 2000a, 2000b) and there is some evidence from both experimental animal models and human studies that vitamin A deficiency may exacerbate anemia through impairment of iron metabolism (Semba and Bloem 2002). These micronutrient interactions strongly argue for multiple micronutrient fortification.

Because salt is consumed daily at fairly steady levels even by population groups in poor remote areas, it could be a promising food vehicle for fortification with iodine, iron (Fe), and vitamin A. However, fortification of salt with all 3 micronutrients together is technically challenging. Most studies with salt dual-fortified with Fe and iodine reported unacceptable color changes and iodine losses due to interactions of the iron compound with the iodine and salt impurities (Diosady and others 2002; Madhavan Nair and others 1998; Sivakumar and others 2001; Venkatesh Mannar and Diosady 1998; Wegmuller and others 2003). To prevent the reaction of Fe and iodine with the salt and with each other, a barrier could be formed by encapsulation. However, a recent stability study of salt dual fortified with iodine and 19 different encapsulated and nonencapsulated Fe compounds in West and North Africa suggested that currently available encapsulation techniques do not allow successful
salt fortification. However, iodized salt fortified with nonencapsulated small particle size ferric pyrophosphate with a mean particle size of 2.5 μm resulted in acceptable color change and iodine stability (Wegmuller and others 2003), and was effective in improving Fe and iodine status in children (Zimmermann and others 2004b). Adding vitamin A to salt is particularly challenging, due to its fat solubility and its instability to oxygen and other oxidizing agents, particularly when it is exposed to light and heat at the same time (BASF 2001). Encapsulation of vitamin A and iodine in an edible fat matrix could protect them from interactions with salt impurities, light, air, and moisture. Fortification of salt with a single dry mix containing iodine, Fe, and vitamin A would be simpler and less expensive compared to separate addition of the 3 micronutrients to different food vehicles.

Spray cooling was chosen for the production of the microcapsules because of the desirable coating characteristics produced by the technique, and because spray cooling is currently the least expensive encapsulation technology (Gouin 2004). Fully hydrogenated palm fat was chosen as coating material due to its high melting point (63°C) which may allow the sprayed capsules to resist high temperatures encountered during storage and transport of salt in tropical climates and due to its hydrophobic properties which prevent the entrance of water and reduce reactions between different core ingredients. In addition fat is an ideal matrix for fat-soluble vitamin A due to the stabilization and the delay of oxidation of the vitamin (Dary and Mora 2002).

For the production of the microcapsules ferric pyrophosphate was chosen as iron source because cool spraying of the better bioavailable compounds ferrous sulfate and ferrous fumarate in palm fat resulted in unacceptable color change when microcapsules were added to salt (Jermann 2003). KI03 was chosen over KI due to less reaction with the ferric form of iron and an oily form of vitamin A was used due to its solubility in the fat matrix, its higher stability and lower costs compared to dried forms (Dary and Mora 2002).
Materials and Methods

Microcapsule components

The coating material used for the encapsulation process consisted of fully hydrogenated palm fat with a melting point of 63°C (Nutriswiss, Lyss, Switzerland) and 1% lecithin (Centrolex F-IP soya pure lecithin, Univar AG, Zürich, Switzerland). The lecithin was added to the molten palm fat together with the Fe to reduce the viscosity of the suspension and enable a capsule:substrate ratio of 60:40. Small particle size ferric pyrophosphate (FePP) (~21% Fe) with a mean particle size (MPS) of 2.5 μm (Dr. Paul Lohmann, Emmerthal, Germany) was used. Reagent-grade potassium iodate (KIO₃) (Riedel-de Haen, Hannover, Germany) was chosen as the iodine source. The KIO₃ was ground to a smaller particle size (<10 μm) before spraying using a laboratory bead mill K8 (Bühler, Uzwil, Switzerland). The KIO₃ was ground at 92 °C for about 90 min in molten palm fat at a ratio of 1:2. After grinding, the MPS of the KIO₃ was ≈ 6 μm, measured by laser diffraction spectrometry (Malvern MasterSizer X, Malvern Instruments Ltd, Malvern, UK; lens 45 mm; wavelength 633 nm; output 2 mW). The vitamin A fortificant used was liquid retinyl palmitate 1.7 M IU/g, stabilized with 10 mg of BHT/million IU (BASF ChemTrade GmbH, Burgbernheim, Germany).

Production of the microcapsules

The particle size of the microcapsules was set considering the following factors: 1) the average particle size of the Moroccan salt (approximately 1.5 mm); a MPS for the microcapsules of ≈ 100 μm was chosen to reduce segregation in the salt; 2) the need to maintain an adequate volume-surface ratio in order to protect the ingredients, but not interfere with release kinetics of the nutrients from the capsules to maintain bioavailability.

A specifically designed stainless steel spraying tower was manufactured by the mechanical workshop of the Food Processing Laboratory at the Institute of Food Science and Nutriton, ETH Zürich. A two-fluid nozzle with hot air as second medium from SSCO-Spraying Systems (Pfäffikon SZ, Switzerland) was used for the atomization. (Specification: SUE 28B: fluid cap PF 35100, air cap 122281-60° (spraying systems); bore width of the fluid cap: originally 0.95, widened to 1.1; bore
width of the air cap: 2.0; spraying angle: 60°). Tubes transporting the fat suspension to the spraying tower were double liner tubes and the outer liner was heated with water of about 90 °C from a water bath to avoid blockage due to solidification of the palm fat at room temperature.

Ferric pyrophosphate with MPS of 2.5 μm (40% w/w) and lecithin (1% w/w) were stirred into molten palm fat (80-90 °C) and than filled into a heated tank above a rotary pump (NMOG 322, ibex pumps, Eastbourne, England). It was constantly stirred with a vibro-mixer (Chemap E1, Chemap, Volketswil, Switzerland) to avoid sedimentation of the Fe particles and then the iodine particles and retinyl palmitate were added and mixed. The mixture contained the added functional components at a ratio of Fe:iodine:retinol of 100mg:2mg:5mg per g mixture. To minimize losses of iodine and retinol due to heat, light and/or oxidation, once these ingredients were added, the final suspension was immediately sprayed into the pre-cooled spraying tower through the two-fluid nozzle with heated air of about 90 °C as the second medium. The pump pressure was set at 1 bar and the air pressure for the nozzle at 2.5 bar. Liquid nitrogen was sprayed into the middle zone of the tower to cool and rapidly solidify the atomized particles. They were collected at the bottom of the tower in a drawer containing a polystyrene recipient laid out with a plastic foil. The capsule:substrate ratio of the produced microcapsules was 60:40.

**Viscosity experiments**

The effect of varying processing parameter conditions on the viscosity of the molten palm fat suspension was studied. This included: 1) suspensions containing 25% (w/w) small particle size FePP with a MPS of 2.5 μm heated to different temperatures (65, 68, 71, 74, 80 °C) and sheared with increasing shear rates ($10^{-1} - 10^3$ s$^{-1}$); 2) suspensions containing 0.5% lecithin (Centrolex F-IP soya pure lecithin, Univar AG, Zürich, Switzerland) or 0.5% polyglycerol polyricinoleate (PGPR) (Danisco Ingredients, Copenhagen, Denmark) in addition to the 25% Fe particles sheared at 70 °C in the shear rate range of $10^{-1} - 10^3$ s$^{-1}$; and 3) suspensions containing varying capsule:substrate (FePP) ratios, with and without the 2 emulsifiers (Lecithin, PGPR) in varying concentrations sheared in the same shear rate range. Viscosity was measured using a Physica MCR-300 rheometer with a Peltier-heated double-gap Couette geometry (Paar Physica, Stuttgart, Germany).
**Particle size measurements**

The particle size distribution of the microcapsules after spraying was measured in air flow by laser diffraction using a Sympatec Helos (Bühler AG, Uzwil, Switzerland). The number distribution $Q_0(x)$ was calculated and the values for $x_{10.0}$ (the diameter of 10% of the number of particles is smaller than $x$), $x_{50.0}$ (the diameter of 50% of the number of particles is smaller than $x$) and $x_{90.0}$ (the diameter of 90% of the number of particles is smaller than $x$) were read from the size distribution. The microcapsule morphology was determined by optical inverse microscopy (Nikon Diaphot TMD, Nikon Corporation, Tokyo, Japan) and by scanning electron microscopy (Hitachi S900, Hitachi, Tokyo, Japan).

**Salt fortification with the microcapsules in Morocco**

The salt fortification level chosen for the Fe compound was equivalent to 1 mg of Fe as ferrous sulfate per gram salt, and was based on: 1) the estimated relative bioavailability of the FePP (approximately 50%) compared to ferrous sulfate (Hurrell 2002b); 2) previous studies of daily salt (approximately 10 g/d) and iron (approximately 12 mg/d, 97% as non-heme Fe from a highly inhibitory diet) intake in Moroccan school children (Zimmermann and others 2003; Zimmermann and others 2004a); and 3) a goal of supplying roughly the estimated daily iron requirement of $\approx$ 8 mg for this age group (Institute of Medicine 2001). Therefore, ferric pyrophosphate was added at a level of 2 mg Fe/g salt. The fortification level for KIO$_3$ was based on: 1) salt intake in Morocco (Zimmermann and others 2003); 2) supplying the estimated daily requirement of 120-150 $\mu$g/d for children 9-18 y old (Institute of Medicine 2001) and 3) anticipation of losses (approximately 50%) during spraying, storage and cooking (WHO/UNICEF/ICCIDD 1996). Therefore KIO$_3$ was added in the double amount at spraying, assuming a final iodine content in the fortified salt of about 20 $\mu$g/g salt. The vitamin A fortification level was based on: 1) salt and vitamin A intake (approximately 250 $\mu$g RAE/d) in Morocco (Zimmermann and others 2003; Zimmermann and others 2004a); 2) estimated losses during spraying (approximately 50%); and 3) on the estimated daily requirement of 400-600 $\mu$g for children 4-13 y old (Institute of Medicine 2001) and on the maximum safe intake of 3000 $\mu$g retinol equivalents per day for pregnant women (WHO/MI 1998). Therefore, vitamin A was
added at the double amount at spraying to achieve a final concentration of about 50 μg vitamin A/g salt.

Local Moroccan salt prepared from drying ponds fed by a salty spring was used to prepare the fortified salts. The salt was unwashed and unground, and has a moisture content between 1-4% depending on the season. To prepare the iodized salt (IS), iodine was added to the salt as reagent-grade KIO₃ (Sigma & Aldrich, Buchs, Switzerland) at a level of 25 μg iodine/g salt. To prepare the triple-fortified salt (TFS), salt was fortified with the microcapsules at a level of 2 mg Fe/ ≈20 μg iodine/ ≈50 μg vitamin A per g salt. The compounds were mixed into the salt in two steps. First, a concentrated 2-kg premix of the salts was prepared in a small electric rotating drum mixer (RRM MINI 80, Engelsmann, Ludwigshafen, Germany) that mixed at 26 rpm for 10 min. The mixture was then transferred to a larger barrel mixer (ELTE 650, Engelsmann AG, Germany), 4 more kg of salt were added, and further mixed at 30 rpm for 5 min.

Iodized salt and salt fortified with the microcapsules were stored as 2-kg batches in closed transparent low-density polyethylene bags for 6 mo in a clean, ventilated room out of direct sunlight. At mixing and after 2, 4, and 6 mo of storage, aliquots of 30 g (n = 3/salt) were taken and placed in tightly sealed plastic containers, wrapped with aluminum foil and frozen until analysis. Iodine concentration was measured in the IS and TFS and vitamin A concentration in the TFS. The color of the two fortified salts was determined and the color of TFS compared to that of IS. Iron content was verified at mixing.

Four commonly-consumed northern Moroccan meals—couscous (semolina), white bread, bissara (fava bean-based soup) and chaaria (semolina noodles in milk)—were prepared with both iodized salt and salt triple fortified with the microcapsules. Three of the meals (couscous, bread, chaaria) have a neutral taste and smell, as well as a pale color. The sensory tests were carried out using the triangle test (Meilgaard and others 1999) and a non-trained panel of 18 local adults per meal. The panel was asked to taste each of the three meals and to select the sample that differed from the other 2. To estimate the overall acceptability of the salt 50% of the households using
either the iodized \((n = 41)\) or triple fortified salt \((n = 30)\) were interviewed about the color and taste acceptability after 10 mo of salt consumption.

**Laboratory analyses**

The fortification level of iron in the triple fortified salt at mixing was analyzed by the standard addition method using flame atomic absorption spectroscopy (SpectrAA-400, Varian, Mulgrave, Australia) after sample dissolution and filtration. Iodine content of the IS was measured after salt aliquots were dissolved in deionized water in a ultrasound bath (Branson 5210, Branson Ultrasonic Corp., Danbury, Conn., U.S.A.) using a modification of the Sandell-Kolthoff reaction originally described for the determination of urinary iodine (Pino and others 1996). This method could not be used for the TFS due to incomplete digestion of the microcapsules, so iodine content of the TFS was measured using inductively coupled plasma-mass spectrometry (Haldimann and others 2003).

Retinyl palmitate was extracted with hexane from the fortified salt. Samples were filtered and measured at an extinction wavelength of 331.6 nm and an emission wavelength of 481 nm by fluorescence spectrophotometry (Luminescence Spectrometer LS 50B, Perkin Elmer, Wiltshire, England) using an external calibration curve.

Color was determined by colorimetry (Chroma-Meter CR-310, Minolta AG, Dietikon, Switzerland) as described in an earlier publication (Wegmuller et al., 2003) using the Hunter Scale. The color of the DFS and IS at each time point was compared to IS at baseline and color lightness \((L\)-value) and color difference \((\Delta E)\) was calculated.

**Statistical analysis**

Data processing and statistics were done using Excel (XP 2002, Microsoft, Seattle, WA, USA). Colorimetry results and iodine and vitamin A content were expressed as means (SD) for IS and TFS at 0, 2, 4, and 6 mo of storage. A two-factor repeated-measures ANOVA was done to compare effects of the fortification \(\times\) time interaction on salt color and iodine content. If the interaction effect was significant \((P < 0.05)\), \(t\) tests between groups and paired \(t\) tests within groups over time were done.
Results and Discussion

Properties of the spraying suspension

Figure 1 shows the viscosity function of a palm fat suspension containing 25% (w/w) FePP (MPS 2.5 μm) at different temperatures as a function of the shear rate. At low shear rates viscosity is more temperature dependent than at high shear rates. At shear rates of \( \dot{\gamma} \leq 200 \text{ s}^{-1} \) viscosity increased with temperature. At shear rates of \( \dot{\gamma} \geq 200 \text{ s}^{-1} \) this phenomenon reversed and viscosity decreased with increasing temperature. A possible explanation of this flow behavior can be given if the competition between structure forces (interparticle forces) and hydrodynamic flow forces is considered. At low shear rates the structure forces dominate the flow forces. In the continuous fat phase the particles will experience strong attractive interparticle forces due to their hydrophilic surfaces. This will lead to particle agglomeration. Consequently, temperature increase leads to enhanced particle agglomeration and the related entrapment of free fluid (melted fat) in the voids and pores of the particle agglomerates causes an increase in the effective volume fraction. As consequence, the viscosity increases. In the high shear rate domain (here \( \dot{\gamma} \geq 200 \text{ s}^{-1} \)) the hydrodynamic forces dominate the structure forces leading to de-agglomeration of the structure. As consequence, only the fluid viscosity which reduces with increased temperature is relevant. This was confirmed with the small particle size FePP (MPS ≈ 2.5 μm), showing a quick rise of viscosity when it was mixed into molten palm fat. It was not possible to produce a suspension containing more than 25% (w/w) of the iron compound (Windhab 2000).

The viscosity functions of suspensions containing 25% (w/w) FePP (MPS = 2.5 μm) with and without the addition of an emulsifier (0.5% lecithin or 0.5% PGPR) were compared. As shown in Figure 2, at low shear rates samples containing emulsifiers had lower viscosity, with PGPR showing a larger reduction in viscosity compared to lecithin. At high shear rates (e.g. during spraying), the viscosity of the samples with emulsifiers was also lower than those without emulsifier, while the viscosity of the 2 samples with emulsifiers was comparable. These findings indicated the benefit of emulsifier addition to the palm fat suspension, allowing the substrate:capsule ratio to be maximized. Varying amounts of lecithin were added to increase the solid content.
of the suspension and thereby reduce the amount of capsule material and costs at the same time. A Finally, a sprayable suspension containing 40% FePP was achieved using 1% lecithin; and a final capsule:substrate ratio of 60:40. A decrease in viscosity in the case of emulsifier addition can be explained by the emulsifier functionality which acts in modifying the interparticle forces and as a spacer between particles and thereby reduces agglomeration (Hugelshofer 2000). The emulsifier may also stabilize specific crystal forms which can affect the diffusion rate of water through the matrix (Gouin 2004).
Figure 1 - Viscosity function of a palm fat suspension containing 25% (w/w) ferric pyrophosphate (MPS ≈ 2.5 μm) at different temperatures.
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Figure 2 - Viscosity function of a palm fat suspension containing 25% (w/w) ferric pyrophosphate (MPS ≈ 2.5 μm) with the addition of 0.5% lecithin, 0.5% PGPR and without emulsifier at constant temperature of 70°C

Particle size and morphology of microcapsules

Table 1 shows the results of the particle size analysis of two batches of sprayed microcapsules. Capsule size ranged from several microns up to several hundred microns. Comparing the distribution of the two sprayed batches, values for \( x_{10.0} \), \( x_{50.0} \) and \( x_{90.0} \) were comparable, with the MPS of 138 and 132 μm, respectively, indicating a good reproducibility. This MPS was close to the desired maximum MPS of ≈ 100 μm, chosen to reduce segregation of microcapsules in the fortified salt. Spray cooling has the advantage that particles of a wide particle size range can be produced (Gouin 2004) and therefore microcapsules can be adapted to the local salt of different countries or to other food vehicles. However, the production process should be improved to produce a narrower particle size range or the capsule product should be classified.
Table 1 - Particle size distribution of two batches of microcapsules

<table>
<thead>
<tr>
<th></th>
<th>$X_{10.0}$</th>
<th>$X_{50.0}$</th>
<th>$X_{90.0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>35.2</td>
<td>138.1</td>
<td>305.2</td>
</tr>
<tr>
<td>Batch 2</td>
<td>28.9</td>
<td>132.3</td>
<td>428.7</td>
</tr>
</tbody>
</table>

$^1$ The diameter of 10% of the number of particles is smaller than x

Figure 3 shows a light microscope image of the microcapsules (100 x magnification). The sprayed particles are of spherical shape with rather smooth surface, which is typical for spray cooled or spray chilled products because there is no mass transfer by evaporation as in spray drying (Shahidi and Han 1993). The image demonstrates the relatively wide particle size range and the presence of agglomerates likely due to insufficiently solidified particles during settling in the spraying tower. Figure 4 shows a SEM micrograph of the surface of microcapsules at two different magnifications. Fat crystals of the palm fat can be observed on the capsule surface. Small capsules show fewer fat crystals on the surface than larger ones most probably due to shorter solidification time for small capsules. To visualize the inner structure microcapsules were broken, and as shown in Figure 5, the large crystals of the palm fat can be recognized on the surface of the cross section. The individual nutrient components are not visible on the surface or in the cross section of the broken capsules. Techniques to visualize the distribution of the different nutrients in and on the surface of the capsule are required for more detailed characterization of the capsules.
Figure 3 - Sprayed microcapsules analyzed by light microscopy (100-times magnified)

Figure 4 - Surface of microcapsules analyzed by SEM micrographs: A) 700-times magnified, picture width: 154 μm and B) 5'000-times magnified, picture width: 21.6 μm
Acceptability and sensory testing

There were no differences detected by the panelists using triangle tests in the four meals fortified with IS and TFS. After 10 months of household salt use (IS or TFS), the head of household was interviewed about the acceptability of the salt. About half of the households used IS (n = 41) and the other half TFS (n = 30) for cooking. There was no difference in the use of the salt, in color acceptability, in salt taste in foods and in overall acceptability between households using IS or TFS. A slight color change, mainly in egg and milk dishes, was observed by 16% of the IS and 32% of the TFS heads of household. However, this slight color change did not affect overall acceptability and was not detected in the triangle tests also performed with dishes containing milk or eggs.
Stability and bioavailability of microcapsules

Table 2 shows the color and iodine content of IS and TFS, as well as the retinol content of the TFS at baseline and after storage for 2, 4, and 6 mo. Color determination showed no significant difference between IS and TFS in color lightness (L-value), and no significant color change in the IS and the TFS over 6 mo of storage. The light beige color of the TFS compared to the milky white color of IS resulted in an absolute color difference (ΔE) of ≈ 9. Although Wegmüller and others (2003) reported fortified salts with ΔE < 10 compared to IS were generally considered acceptable during interviews at local African markets, an improvement in salt color could be a future aim in the refinement of the encapsulation process. Ferric pyrophosphate with a whiter color than the one used (for example, reagent-grade material) would be a possibility, but would be more expensive and more difficult to produce on a large scale. Since fats crystallize in various forms that exhibit very different crystal sizes, hydrophobicities and densities (Gouin 2004), refinement of the crystallization during production and storage may also help in preventing reactions of the Fe compound resulting in discoloration.

The spraying process resulted in iodine losses of approximately 40%. Losses after storage for 6 mo were additionally about 20% in both salts, with no significant differences comparing TFS to IS. During spraying approximately 30% of the retinol was lost. The retinyl palmitate used in the experiments was surprisingly stable during storage with small losses of about 12% after 6 mo, presumably due to the hydrogenated fat acting as an excellent barrier to oxygen. These results however indicate that retinol and iodine losses during production of the capsules are relatively high, and the encapsulation process could be further improved to prevent these losses, particularly for retinol, which is expensive. Producing the microcapsules in oxygen free area and without direct light could be a next step.
Table 2 - Color, iodine, and vitamin A concentration in iodized salt (IS) and triple fortified salt (TFS) after storage for 0, 2, 4 and 6 months and fortification level before storage

<table>
<thead>
<tr>
<th>Fortification level</th>
<th>Vitamin A</th>
<th>Iodine</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/g salt</td>
<td>µg/g salt</td>
<td>Lightness</td>
</tr>
<tr>
<td>TFS IS TFS IS TFS TFS/IS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 25 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mo</td>
<td>69.7 ± 7.1</td>
<td>27.4 ± 3.8</td>
<td>25.7 ± 3.0</td>
</tr>
<tr>
<td>2 mo</td>
<td>74.5 ± 3.2</td>
<td>18.6 ± 2.1</td>
<td>22.2 ± 6.1</td>
</tr>
<tr>
<td>4 mo</td>
<td>69.4 ± 2.7</td>
<td>21.0 ± 3.1</td>
<td>26.5 ± 3.7</td>
</tr>
<tr>
<td>6 mo</td>
<td>61.2 ± 4.5</td>
<td>19.4 ± 1.4</td>
<td>20.6 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD (n=3)  
<sup>2</sup> Lightness scale: 0=black, 100=white  
<sup>3</sup> ∆E<sub>ab</sub> absolute color difference between TFS and IS  
<sup>4</sup> Iodine and vitamin A added before spraying for TFS and I added at mixing for IS  
<sup>5</sup> Significant main effect of fortification, P<0.001 (ANOVA)  
<sup>6</sup> Significant main effect of time, P<0.001 (ANOVA)

Besides the good stability of the microcapsules, the bioavailability of nutrients from the capsule and thereby its potential efficacy in human nutrition is an important consideration. Spray cooling is typically referred to as 'matrix' encapsulation, because the particles are more adequately described as aggregates of active ingredient particles buried in the fat matrix, while 'true' encapsulation is usually reserved for processes leading to core/shell type of microcapsules. A matrix encapsulation process leaves a significant proportion of the active ingredients lying on the surface of the microcapsules or sticking out of the fat matrix, thus having direct access to the environment and generally releasing their content easily (Gouin 2004). However, a strong binding of the ingredient to the fat matrix can prevent the release of the ingredient even though the fat matrix is melted and/or damaged during processing (Gouin 2004). In a series of rat studies (Hurrell 1985; Hurrell and others 1989) the relative bioavailability (RBV) of encapsulated ferrous sulfate at a 40:60 capsule:substrate ratio was comparable to that of nonencapsulated ferrous sulfate. A study in children consuming salt fortified with ferrous sulfate encapsulated in partially hydrogenated vegetable oil (ratio 50:50) reported the encapsulated iron was highly effective in reducing the prevalence of IDA (Zimmermann and others 2003), suggesting encapsulation with a fat matrix had little or no effect on bioavailability. However, studies with encapsulated ferrous sulfate in hydrogenated soybean oil and
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ferric ammonium citrate in hydrogenated palm oil showed a reduction of RBV of about 20-25% at a capsule:substrate ratio of 60:40 (Zimmermann 2004). In a recent rat study, comparing the RBV of small particle size FePP encapsulated with the method described in this paper (fully hydrogenated palm oil at a capsule:substrate ratio of 60:40) to the nonencapsulated compound, a 40% decrease in RBV was found (Wegmuller and others 2004). However, small particle size FePP encapsulated together with iodine and retinyl palmitate in palm fat (60:40 capsule:substrate ratio) was recently tested in a human efficacy trial of fortified salt in Morocco, and was shown to be highly efficacious in improving iron status (Zimmermann and others 2004a). Nevertheless, new WHO guidelines (WHO 2005) recommend a capsule:substrate ratio of ≤ 50:50 due to possible losses of bioavailability with greater ratios.

Although ferric pyrophosphate with a particle size ≥10 μm has a relative bioavailability of only 20-70% compared to ferrous sulfate (Hurrell 2002a; Hurrell 2002b; Wegmuller and others 2004), reducing its particle size to 0.5 μm (SunActive Fe, Taiyo Kagaku Co., Ltd., Mie, Japan) increases its bioavailability in humans to that of ferrous sulfate (Fidler and others 2004). In a recent rat study the reduction of MPS of ferric pyrophosphate from 21 μm to 2.5 μm resulted in a non significant increase of RBV from 59% to 69% and further reduction to a MPS of 0.5 μm increased RBV to 95%, comparable to ferrous sulfate (Wegmuller and others 2004). Due to the high cost of commercial 0.5 μm FePP which is coated with emulsifiers to make it water dispersible, the 2.5 μm compound was used in the present studies. The lower RBV of this compound compared to ferrous sulfate (approximately 70% in rats) was taken into account during microcapsule preparation and salt fortification, with a final amount of 2 mg Fe added per gram salt.

Processing costs to reduce particle size of FePP to 2.5 μm tend to approximately double the cost (Dr. Paul Lohmann, Emmerthal, Germany) and could be a significant barrier for the use of small particle size FePP in fortification programs in developing countries. Additional costs for the encapsulation material as well as production costs must also be considered. If microencapsulation provides the ingredient with a unique property that is not achievable without encapsulation, the cost-in-use is allowed to be slightly higher than that of the nonencapsulated ingredient (Gouin 2004). However,
the price increment for salt fortification, especially with vitamin A, tends to be high due to the low baseline price of the salt. However, in many regions with subsistence farming in Africa and Indonesia, salt is one of few regularly purchased food items and therefore often is the only suitable food vehicle for food fortification (Hess and others 1999; Melse-Boonstra and others 2000; Staubli-Asobayire 2000). Fortification of salt with iodine is highly effective in reducing IDD (WHO/UNICEF/ICCIDD 2001) and some food stuffs, such as oil (Favaro and others 1992; Johnson 1997), margarine (Johnson 1997; Solon 1997a; Sridhar 1997), monosodium glutamate (Muhilal and others 1988; Solon and others 1985), cereal flours (Chavez 1997; Solon 1997b) and sugar (Mora and others 2000) have been shown to be efficacious in reducing vitamin A deficiency. But these fortification vehicles are often not widely distributed or not consumed by the neediest population groups. Successful iron fortification has been demonstrated with infant cereals and formulas (Walter and others 1990; Walter and others 1998), sugar (Viteri and others 1995), curry powder (Ballot and others 1989), and fish sauce (Thuy and others 2003), but not yet with cereal and corn flours (Hurrell 2002b). However, the same problem of limited access by the neediest applies to Fe fortification with these food vehicles. Thus, triple fortification of salt with a single compound containing all three micronutrients could be a useful and simple new fortification strategy.

**Conclusions**

Spherical microcapsules containing FePP, iodine, and vitamin A for use in salt fortification were successfully produced by spray cooling. Although iodine and vitamin A losses during production of the microcapsules were relatively high, losses during storage after addition to salt were low. Through the encapsulation vitamin A added as retinyl palmitate was dissolved in the fat matrix before spraying and showed excellent stability in salt. Iodine and color stability of salt fortified with the microcapsules were acceptable. By using a small particle size FePP (MPS = 2.5 μm) and a higher fortification level the low bioavailability of regular FePP was compensated. A study with school age children consuming salt fortified with the microcapsules showed high efficacy of all 3 micronutrients in Morocco (Zimmermann and others 2004a). These findings indicate that triple fortification of salt with a single compound containing all three micronutrients could be a useful and simple new fortification strategy. However,
Microcapsules containing iron, iodine, and vitamin A process parameters and raw materials should be further optimized in order to 1) decrease the capsule:substrate ratio of 60:40 to minimize possible losses in bioavailability of the encapsulated nutrients; 2) minimize color changes due to the off-white ferric pyrophosphate and not tight enough capsules and 3) reduce losses of iodine and especially of retinyl palmitate during spray cooling 4) reduce costs since this could be a significant barrier in fortification programs in developing countries.

Acknowledgments

This study was supported by the Swiss Federal Inst. of Technology (Zürich, Switzerland). We thank Daniel Kiechl (Swiss Federal Inst. of Technology, Switzerland) for technical assistance in building and improving the spraying tower and in spraying the encapsulated compound; Dr. Paul Lohmann GmbH (Emmerthal, Germany) for providing the small particle size ferric pyrophosphate and BASF ChemTrade GmbH (Burgbernheim, Germany) for providing the retinyl palmitate; Oskar Krois and Priscilla Fässler (Bühler AG, Uzwil, Switzerland) for the help in the milling process and the particle size measurements; Max Haldimann (Swiss Federal Office of Public Health, Switzerland) for ICP-MS measurements of the triple fortified salt; Rabie Rahmouni (Brikcha, Morocco) and Christophe Zeder (Swiss Federal Inst. of Technology, Switzerland) for assistance in sampling and mixing; Fabian Rohner (Swiss Federal Inst. of Technology, Switzerland) for assistance in the sensory testing.
References


Microcapsules containing iron, iodine, and vitamin A


Particle size reduction and encapsulation affect the bioavailability of ferric pyrophosphate in rats

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Abstract

Particle size is an important determinant of Fe absorption from poorly soluble Fe compounds in foods. Decreasing the particle size of elemental iron powders increases their absorption. The effect of a reduction in particle size on the bioavailability of ferric pyrophosphate (FePP) is unclear. Encapsulation of iron compounds for food fortification may protect against adverse sensory changes, but at the same time may reduce bioavailability. The hemoglobin (Hb) repletion method in weanling Sprague-Dawley rats (n=100) was used to compare the relative bioavailability (RBV) of 4 forms of FePP: 1) regular FePP [mean particle size (MPS) ≈ 21 μm]; 2) MPS ≈ 2.5 μm; 3) MPS ≈ 2.5 μm encapsulated in hydrogenated palm oil; and 4) MPS ≈ 0.5 μm with emulsifiers. The RBV compared with ferrous sulfate was calculated by the slope-ratio technique. The RBV was 43% for encapsulated MPS ≈ 2.5 μm, significantly lower than the other FePP compounds (P < 0.05), 59% for the regular FePP, and 69% for MPS ≈ 2.5 μm, not different from each other but significantly lower than ferrous sulfate (P < 0.05), and 95% for emulsified MPS ≈ 0.5 μm, comparable to ferrous sulfate. Encapsulation of FePP with hydrogenated palm oil at a capsule:substrate ratio of 60:40 decreased RBV. Particle size reduction increases the RBV of FePP and may make this compound more useful for food fortification.

Key words: ferric pyrophosphate, iron, bioavailability, particle size, encapsulation
Introduction

Iron (Fe) deficiency anemia (IDA) is a major global public health problem, affecting primarily young women, infants and children (1). Although food fortification with Fe may be an effective strategy to control IDA, successful Fe fortification of foods remains a challenge (2). Water-soluble, highly bioavailable Fe compounds often cause adverse organoleptic changes in foods. Poorly soluble Fe compounds, although more stable in foods, tend to have low bioavailability. Ferric pyrophosphate (FePP) is a white-colored, poorly soluble iron compound that does not cause sensory changes in many difficult-to-fortify food vehicles, including salt (3). However, its low bioavailability, only 30–50% of ferrous sulfate, reduces its nutritional value (4).

Particle size can be an important determinant of Fe absorption from poorly soluble Fe compounds in foods. Decreasing the particle size of elemental iron powders by 50–60%, to a mean particle size (MPS) of 7–10 µm, increases Fe absorption by ~50% in rats (5,6). In a human study, Fe absorption from hydrogen-reduced elemental Fe with particle sizes between 5 and 10 µm was comparable to that from Fe sulfate (7). Similarly, reducing the particle size of FePP may increase its absorption. Conventional grinding can decrease the MPS of FePP to 2–3 µm. Further reduction in MPS to <1 µm is possible by generating FePP particles in aqueous solutions and adding emulsifiers to prevent agglomeration (8). In a human stable isotope study, the RBV of a dispersible FePP with a MPS of 0.5 µm was comparable to that of ferrous sulfate (9). In a recent intervention trial in Moroccan children with dual-fortified salt, FePP with a MPS of 2.5 µm demonstrated high bioavailability and efficacy (10).

Fe encapsulation has the potential to help overcome several major challenges in Fe fortification of foods. It may decrease unwanted sensory changes and reduce interactions of Fe with other food components (2). However, encapsulation can adversely affect Fe bioavailability. In rat studies, encapsulation of ferrous sulfate in hydrogenated soybean oil and ferric ammonium citrate in palm oil at a ratio of capsule:substrate of ≥60:40 decreases Fe RBV by ~20% (11).

To determine the potential effects of particle size reduction and encapsulation on the bioavailability of FePP, we tested the RBV of 3 FePP compounds with varying...
particle sizes [regular (MPS ≈ 21 µm), ≈ 2.5 µm, ≈ 0.5 µm] and of one encapsulated FePP (60:40 capsule:substrate) using the hemoglobin (Hb) repletion assay in rats.

Materials and Methods

Iron compounds
The iron compounds tested are shown in Table 1. The encapsulated FePP was produced in our laboratory at the Swiss Federal Institute of Technology Zurich by spray cooling. The capsule was made of hydrogenated palm oil (Nutriswiss) containing 1% lecithin (Loders Croklaan). The palm oil was heated to ~85°C and a suspension containing the FePP was made. This was then passed through a screw pump (Scheerle) into a stainless steel spraying tower, atomized using air as the second medium, and cooled with liquid nitrogen.
TABLE 1: Iron compounds used to fortify repletion diets

<table>
<thead>
<tr>
<th>Iron compound</th>
<th>Capsule material/Additives</th>
<th>Iron %</th>
<th>Particle size μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous sulfate (dried)(^3)</td>
<td>None</td>
<td>29</td>
<td>70% &lt; 100(^1)</td>
</tr>
<tr>
<td>(3590540)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric pyrophosphate(^3)</td>
<td>None</td>
<td>21</td>
<td>80% &lt; 100(^1)</td>
</tr>
<tr>
<td>(3043420)</td>
<td></td>
<td></td>
<td>d(_{10})=4.2(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(_{50})=21.1(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(_{90})=105.2(^2)</td>
</tr>
<tr>
<td>Ferric pyrophosphate(^3)</td>
<td>None</td>
<td>21</td>
<td>≈2.5 (mean)(^1)</td>
</tr>
<tr>
<td>(3043448)</td>
<td></td>
<td></td>
<td>d(_{10})=0.4(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(_{50})=2.5(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(_{90})=6.1(^2)</td>
</tr>
<tr>
<td>Ferric pyrophosphate(^4)</td>
<td>Fully hydrogenated palm oil, lecithin (capsule:substrate ratio = 60:40)</td>
<td>8.4</td>
<td>≈2.5 (mean)(^1)</td>
</tr>
<tr>
<td>(3043448)</td>
<td></td>
<td></td>
<td>d(_{10})=0.4(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(_{50})=2.5(^2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(_{90})=6.1(^2)</td>
</tr>
<tr>
<td>Ferric pyrophosphate(^5)</td>
<td>Dextrin, glycerol esters of fatty acids, sodium chloride, enzymatically hydrolyzed lecithin</td>
<td>8</td>
<td>≈0.5 (mean)(^1)</td>
</tr>
<tr>
<td>(SUNACTIVE Fe-P80)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Particle size of the iron from the specification of the producer.
\(^2\) Particle size of the iron measured in our laboratory by laser diffraction spectrometry (Malvern Master Sizer X, Renens-Lausanne, Switzerland).
\(^3\) Produced by Dr. Paul Lohmann GmbH KG, 31857 Emmerthal, Germany.
\(^4\) Produced by Dr. Paul Lohmann GmbH KG, 31857 Emmerthal, Germany and the Swiss Federal Institute of Technology, Zurich, Switzerland.
\(^5\) Produced by Taiyo Kagaku Co. Ltd., Yokkaichi, Mie, Japan.

**Animals and diets**

The Veterinary Office of the Canton of Zurich, Switzerland, approved the study. The bioavailability of the Fe compounds was determined by the Hb repletion method (12–14). Two levels of dietary iron were used for each compound. Male weanling Sprague-Dawley rats (\(n = 100\); Charles River) at 21 days of age were housed individually in plastic cages with grated stainless steel floors, and kept under controlled conditions with a daily 12-h light:dark cycle. Millipore water (Milli-Q UF Plus, Millipore) was provided for all rats ad libitum throughout the whole study. All of the rat diets used in the study were prepared by Dyets. Rats consumed an iron-deficient diet [~2 mg Fe/kg AIN-93G purified rodent diet (15)] ad libitum for 24 d. After this depletion period, the rats were weighed and blood was collected by tail incision (16) into EDTA-coated tubes for Hb determination. Rats with Hb values between 30 and 60 g/L (mean ± SD, 44 ± 5 g/L; range 33–57 g/L) were randomly assigned to 11
groups of 8 rats. The rats consumed the same Fe-deficient diet (AIN-93G diet), but fortified with 1 of the 4 FePP compounds (each at \( \sim 10 \) or 20 mg Fe/kg diet), ferrous sulfate (FeSO\(_4\) \( \cdot \) H\(_2\)O; at \( \sim 10 \) or 20 mg Fe/kg diet), or no added Fe (\( \sim 2 \) mg Fe/kg diet) for 14 d ad libitum. Other than their Fe content, the diets were equivalent and conformed to the recommendations for AIN-93 purified diets (15). The Fe content of all diets was verified by atomic absorption spectroscopy (SpecrAA-300/400 with GTA-96 Graphite Tube Atomizer, Varian Techtron). Individual food consumption was recorded daily throughout the repletion period. After the repletion period, rats were weighed, blood was collected by tail incision for Hb determination, and the rats were killed.

**Laboratory analysis**

Hb concentration was measured in triplicate in whole blood with a commercial kit (Hb MPR 3, ref 124729, Roche Diagnostics) using the cyanmethemoglobin method (17), and commercially available control material (Digitana AG).

**Slope-ratio modeling and statistical analysis**

Data processing and statistics were done using SPSS 12.0. Using the slope-ratio method, the bioavailability of each test Fe source relative to FeSO\(_4\) was calculated by comparing change in Hb \( [g/(L \cdot 14d)] \) with the following: 1) the Fe level in the diet; and 2) the absolute Fe intake (µg/d) (18,19). The slope of the responses for each dietary Fe source was calculated by using a common-intercept multiple linear regression model with the 'no added Fe' group serving as the blank. Linearity of the regression curves was ascertained for each Fe source separately. Tests were conducted to determine whether the mean of the blank differed significantly from the common intercept for the 4 Fe compounds. Tukey's method was applied to test whether the slopes of the Fe compounds were significantly different from that of FeSO\(_4\) and from each other (P < 0.05). Using Fieller's method (18), 95% CIs for relative bioavailability were obtained. Fe intake, body weight gain, and Hb change during the repletion period were expressed as mean ± SEM, \( n = 8/\)Fe compound and fortification level.
Results

Fortification level, fortification Fe intake, body weight gain, and Hb change during the 14-d repletion period are shown in Table 2. The RBVs calculated using dietary fortification Fe concentration (mg/kg) and Fe intake (µg/d) are shown in Table 3. In both models, the regression lines for the Fe compounds did not significantly depart from linearity and the mean of the blank was not different from the common intercept. \( R^2 \) for the models using fortification iron and Fe intake were 0.89 and 0.88, respectively, and did not differ.

The RBV of the FePP compounds did not differ using the 2 models. The RBV of the emulsified MPS ≈ 0.5 µm was higher than that of the other FePP compounds (\( P < 0.05 \)), and was not different from ferrous sulfate. The RBV of the regular FePP and the MPS ≈ 2.5 µm was 59 and 69%, respectively, significantly lower than ferrous sulfate and not different from each other (\( P > 0.05 \)). The RBV of the encapsulated MPS ≈ 2.5 µm was 43%, significantly lower than that of the MPS ≈ 2.5 µm in both models and, in addition, significantly lower than the regular FePP when the dietary fortification level model was applied (Table 3). The dose-response curves on the basis of Fe fortification level (mg Fe/kg diet) or Fe intake (µg/d) and on change in Hb concentration (g/L · 14d) are shown in Figure 1.
### TABLE 2: Fortification level, fortification iron intake, body weight gain and change in hemoglobin during repletion period

<table>
<thead>
<tr>
<th>Iron compound</th>
<th>Fortification level</th>
<th>Fortification iron intake</th>
<th>Body weight gain</th>
<th>Hb change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg Fe/kg diet</td>
<td>µg/d</td>
<td>g/14 d</td>
<td>g/(L · 14 d)</td>
</tr>
<tr>
<td>Control (no added Fe)</td>
<td>0</td>
<td>0 ± 0</td>
<td>54.8 ± 3.6</td>
<td>-11.5 ± 1.5</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>10</td>
<td>184.0 ± 5.5</td>
<td>78.8 ± 3.0</td>
<td>14.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>406.0 ± 16.4</td>
<td>96.9 ± 5.5</td>
<td>46.4 ± 1.4</td>
</tr>
<tr>
<td>FePP (d50 = 21 µm)²</td>
<td>10</td>
<td>170.1 ± 7.3</td>
<td>70.7 ± 4.3</td>
<td>4.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>388.0 ± 14.3</td>
<td>85.3 ± 3.8</td>
<td>21.5 ± 2.7</td>
</tr>
<tr>
<td>FePP (d50 = 2.5 µm)²</td>
<td>10</td>
<td>184.1 ± 7.2</td>
<td>77.6 ± 4.4</td>
<td>4.7 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>372.5 ± 10.3</td>
<td>81.6 ± 4.0</td>
<td>28.5 ± 2.2</td>
</tr>
<tr>
<td>FePP enc.³ (d50 = 2.5 µm)²</td>
<td>10</td>
<td>177.5 ± 7.3</td>
<td>70.9 ± 5.4</td>
<td>-0.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>345.0 ± 8.7</td>
<td>74.8 ± 4.9</td>
<td>11.8 ± 3.1</td>
</tr>
<tr>
<td>FePP (mean = 0.5 µm)⁴</td>
<td>10</td>
<td>173.3 ± 5.4</td>
<td>76.3 ± 2.5</td>
<td>12.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>414.5 ± 14.3</td>
<td>95.5 ± 4.1</td>
<td>43.7 ± 2.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8 per tested Fe compound and fortification level.
2 d50: mean particle size measured by laser diffraction spectrometry (Malvern Master Sizer X, Renens-Lausanne, Switzerland).
3 Encapsulated in palm oil.
4 Mean particle size of the iron from the specification of the producer.

### TABLE 3: Relative bioavailability value (RBV) of ferric pyrophosphate compounds compared with ferrous sulfate

<table>
<thead>
<tr>
<th>Iron compound</th>
<th>RBV²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe fortification level</td>
</tr>
<tr>
<td></td>
<td>mg/kg diet</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>100²</td>
</tr>
<tr>
<td>Ferric pyrophosphate (d50 = 21 µm)³</td>
<td>59 (52.68)²</td>
</tr>
<tr>
<td>Ferric pyrophosphate (d50 = 2.5 µm)³</td>
<td>69 (61.78)²</td>
</tr>
<tr>
<td>Ferric pyrophosphate (d50 = 2.5 µm)³ encapsulated in palm oil</td>
<td>43 (35.51)³</td>
</tr>
<tr>
<td>Ferric pyrophosphate (mean = 0.5 µm)⁴</td>
<td>95 (86.104)⁴</td>
</tr>
</tbody>
</table>

1 Values are means (95% CI). Means in a column without a common letter differ, P < 0.05.
2 Bioavailability (RBV) relative to FeSO₄·H₂O (=100), measured by the slope-ratio assay, using either fortification iron concentration in the diet or absolute iron intake (x-axis) vs. repletion in Hb concentration (y-axis) to determine slopes.
3 d50: mean particle size measured by laser diffraction spectrometry (Malvern Master Sizer X, Renens-Lausanne, Switzerland).
Bioavailability of ferric pyrophosphate in rats

Figure 1: Dose-response curves for the Hb repletion assay in depleted rats consuming a Fe-deficient diet or the Fe-deficient diet fortified (10 and 20 mg Fe/kg diet) with ferrous sulfate (Fe sulfate), ferric pyrophosphate with different particle sizes (FePP, 0.5 μm; regular FePP, 21 μm; FePP, 2.5 μm) or with encapsulated 2.5 μm ferric pyrophosphate fortification level (mg/kg diet) or (B) Fe intake (μg/d) and on change in Hb concentration [g/(L·14d)]. Values are mean daily Fe intake ± SD, n = 8 for panel B and mean change in Hb concentration ± SD, n = 8 for panels A and B. The variable “b” indicates the slope of the regression line.

Discussion

Particle size can be an important determinant of Fe absorption from poorly soluble Fe compounds in foods. Decreasing the particle size of elemental Fe powders significantly increases their absorption in rats (5,6) and humans (7). In a recent rat study, the bioavailability of different elemental Fe powders was compared; carbonyl Fe, with a smaller particle size than the other tested compounds, had the highest RBV (20).

FePP has a low-to-medium bioavailability, due to its slow and incomplete dissolution in the gastric juice (4). Human stable isotope studies reported that the RBV of regular FePP is only 30–40% that of ferrous sulfate and ferrous fumarate (21,22). However, several recent studies suggested that decreasing the particle size of FePP may increase its absorption. In a human study, decreasing the MPS of FePP from 8.5 to 6.7 μm increased its RBV compared with Fe sulfate from 36 to 52%, although this was not significant (P = 0.08) (22). In a recent randomized, double-blind intervention
study in Morocco, dual fortification of salt with iodine and FePP with a MPS \(\approx 2.5 \ \mu m\) at a level of 2 mg Fe/g salt was highly efficacious in reducing the prevalence of IDA among schoolchildren (10). In a rat Hb repletion study, the RBV of commercial FePP and the dispersable MPS \(\approx 0.5 \ \mu m\) FePP were found to be 56 and 104% compared with ferrous sulfate (23). In a human stable isotope study, the absorption of dispersable MPS \(\approx 0.5 \ \mu m\) FePP fed in a fortified infant cereal and yoghurt drink was comparable to that of ferrous sulfate (9). However, it is unclear whether the high RBV of this FePP compound is due only to its small particle size or whether the surrounding emulsifiers may also play a role.

We chose the modified AOAC hemoglobin repletion method in rats to evaluate the RBV of the FePP compounds in this study. This method was validated against clinical measurements of Fe absorption in human subjects using radiotracers (14). The findings of the present study are novel in that they suggest decreasing FePP particle size from a MPS \(\approx 21 \ \mu m\) to 2.5 \(\mu m\) to 0.5 \(\mu m\) results in a step-wise increase in absorption. The RBV of the regular FePP compound was 59%, comparable to results obtained in other studies in rats (45–58%) and humans (21-74%) (2,23). Decreasing MPS to \(\approx 2.5 \ \mu m\) increased RBV to 69%; decreasing MPS to \(\approx 0.5 \ \mu m\) significantly increased RBV to 95%, a value comparable to ferrous sulfate.

Our findings also demonstrate that encapsulation of Fe compounds may reduce their bioavailability. In the present study, encapsulation of MPS \(\approx 2.5 \ \mu m\) FePP with hydrogenated palm fat at a capsule:substrate ratio of 60:40 reduced RBV from 69 to 43% \((P < 0.05)\). In a series of rat Hb repletion tests, the RBV of encapsulated ferrous sulfate at a 40:60 capsule:substrate ratio was comparable to that of nonencapsulated ferrous sulfate (24,25). However, the RBV of ferrous sulfate was reduced 20% when encapsulated in hydrogenated soybean oil at a capsule:substrate ratio of 60:40 (11). Similarly, the RBV of ferric ammonium citrate was reduced 25% when encapsulated in hydrogenated palm oil at a 60:40 ratio of capsule:substrate. Our findings support the recent recommendation that the bioavailability of encapsulated Fe compounds should be tested in rat Hb repletion studies before being recommended for use in food fortification (11).
Reducing the particle size of FePP may increase Fe bioavailability and make this compound more useful for food fortification. However, several questions remain. Although particle size reduction to ~ 0.5 μm clearly increases the RBV of FePP, the commercially available compound is prohibitively expensive and patented. It is unclear whether reducing particle size of FePP to 2.5 μm is justified; in the present study, although RBV was increased, the difference was not significant (P = 0.31). Processing costs to reduce particle size are high and could be a major barrier to the use of small particle size FePP in fortification programs in developing countries. If these issues can be resolved, the promise of small particle size FePP is its combination of inert sensory characteristics with good bioavailability.

Acknowledgements

We thank Daniel Kiechl for technical assistance in spraying the encapsulated compound and Luciano Molinari (Children’s Hospital, Zürich, Switzerland) for statistical advice. We thank Taiyo Kagaku Company, Ltd. (Mie, Japan) for providing the MPS ≈ 0.5 FePP, and Dr. Paul Lohmann (Emmerthal, Germany) for providing the remaining Fe compounds.

Literature cited


Salt dual-fortified with iodine and micronized ferric pyrophosphate improves iron status but not hemoglobin in children in Côte d'Ivoire

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Report

This study was supported by the Swiss Federal Institute of Technology (Zürich, Switzerland), the Walter Hochstrasser Foundation (Zürich, Switzerland) and the .
Abstract

Background: Deficiencies of iron and iodine are common in West Africa, and salt is one of very few food vehicles available for fortification.

Objective: We tested the efficacy of salt dual-fortified with iodine and micronized ground ferric pyrophosphate (FePP) in rural, tropical Côte d’Ivoire.

Design: Salt and iron intakes, and iron bioavailability were estimated using 3-day weighed food records in 24 households. Local iodized salt was fortified with 3 mg Fe/g salt as FePP (mean particle size=2.5 μm), and stability, sensory and acceptability trials were done. The dual fortified salt (DFS) was distributed to households and its efficacy compared to that of iodized salt (IS) in a 6-mo, double-blind trial in 5-15 y old iron deficient children (n=123). All children were dewormed at baseline.

Results: After 6 mo, SF, TfR and body iron stores significantly improved in the DFS group compared to IS (P<0.05). Mean body iron (mg/kg) increased from 4.6 to 5.9 in the DFS group vs. 5.5 to 5.6 in the IS group. However, mean hemoglobin and the anemia prevalence did not change in either group. The prevalence of malaria, soil-transmitted helminths, and riboflavin deficiency were 55%, 14%, and 66%, respectively.

Conclusions: In tropical West Africa, low grade salt fortified with micronized FePP increased body iron stores in children but not hemoglobin. Iron utilization may have been impaired by the high prevalence of malaria and concurrent nutrient deficiencies.

Key words: iron, ferric pyrophosphate, deficiency, anemia, salt, iodine, children, Africa
Introduction

In regions of West Africa, 20-38% of school children may suffer from both iron (Fe) and iodine deficiencies (Zimmermann et al., 2000b). In countries, where existing food supplies and/or limited access fail to provide adequate levels of these nutrients in the diet, food fortification is a promising approach. However, there have been no published successful trials of food fortification with Fe in tropical Africa possibly due to other concurrent micronutrient deficiencies and the high prevalence of infection which can reduce Fe absorption and decrease Fe mobilization from stores (Ramakrishnan, 2001; Stoltzfus et al., 2004). Salt is one of very few food items consumed daily by rural African populations even in poor remote areas of subsistence farming (Staubli-Asobayire, 2000). Salt has been effectively iodized in many African countries, and it would be advantageous to use the existing infrastructure for iodization to also fortify salt with Fe. An additional benefit of adding Fe to iodized salt, besides the positive effect of Fe on cognitive development, school performance, immune function and work capacity (FAO/WHO, 2001c), is that iron-deficiency anemia (IDA) impairs thyroid metabolism and reduces the efficacy of iodine prophylaxis in areas of endemic goiter (Zimmermann et al., 2000b; Hess et al., 2002a).

However, when ferrous Fe is added to low-grade iodized salt in developing countries, it causes unacceptable color changes and iodine losses (Sivakumar & Nair, 2002; Wegmuller et al., 2003). One approach is to place a barrier around ferrous Fe by encapsulation. In Morocco, a DFS containing ferrous sulfate encapsulated with hydrogenated oil showed good efficacy, but the salt discolored when the moisture content was high (Zimmermann et al., 2003b). Micronized ferric pyrophosphate (FePP) is a promising fortificant for salt (Wegmuller et al., 2003). It has a white color and although insoluble, FePP with a mean particle size (MPS) of 2.5 μm has a RBV of 69% that of ferrous sulfate in rats (Wegmuller et al., 2004). In Morocco, iodized salt fortified with micronized FePP (MPS of 2.5 μm) showed high efficacy in reducing IDA in children (Zimmermann et al., 2004b). However, conditions are more challenging in tropical Africa, where ambient temperature and humidity is high, salt is typically low-grade, and parasitic infections and nutrient deficiencies are common and may blunt the response to Fe interventions (Gamble et al., 2004).
We fortified local, low-grade iodized salt in Côte d’Ivoire with micronized FePP and tested its stability, organoleptic qualities in traditional meals and acceptability. We measured the efficacy of the DFS to improve iron status in a 18-mo intervention trial in iron-deficient school children in rural Côte d’Ivoire. IS was used as a control.

**Subjects and Methods**

**Study site**

The study site was a rural village in the Dabou district of Côte d’Ivoire, 10 km from the southern coast. The climate is tropical, with temperatures of ≥27°C and a relative humidity of ≥80% much of the year. During the ≈ 6 mo rainy season, the village experiences daily drenching rains. Most of the foods consumed are produced locally, and the staple food is cassava. Plantain, rice, yam and dried, smoked fish are eaten regularly.

**Measurements of food and nutrient intake and calculation of dietary iron bioavailability**

To establish the optimal fortification level for Fe in the salt, 3-day weighed food records were conducted in 24 randomly-selected village households. The records were done on three consecutive days (including 2 week days and one weekend day) during which the households were asked to maintain their usual food habits. In the village, family meals are traditionally eaten from shared bowls. To more accurately estimate individual food intake, during the 3 days of records families ate from individual bowls. Edible portions of all food and beverages were weighed using a Soehnle scale (vera 67002, Soehnle-Waagen GmbH & Co. KG, Murrhardt, Germany) accurate to ± 1 g during preparation and consumption. Salt added during preparation of the meal and at consumption was weighed on a Korona scale (1035550, KORONA-Haushaltswaren GmbH & Co. KG, Langgöns-Niederkleen, Germany) accurate to ± 0.1 g. Meals typically consisted of a cooked staple and a sauce. When the meal was ready the total of the cooked staple and sauce was weighed. All the bowls of each individual household member were distinguished by size and color to facilitate the identification after the meal. After the meal, the food that remained uneaten was weighed and the proportion of staple to sauce was estimated. Subjects
absent during the day were asked about the food and amounts eaten outside the house. If they could not provide sufficient information, they were excluded from the study.

Nutrient intakes (Fe, vitamin C, phytic acid, polyphenols, vitamin A, riboflavin) were calculated using food composition tables (Touriy et al., 1965; FAO, 1968; Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, 1996; Staubli-Asobayire, 2000; Reddy & Sathe, 2002) and food analysis software EBISpro (University of Hohenheim/Stuttgart, Germany). Vitamin A intakes were calculated as retinol activity equivalents (RAEs) using a conversion factor of 12 μg β-carotene to 1 μg retinol for a mixed diet (Institute of Medicine, 2001; West et al., 2002). For β-carotene from palm oil, the standard equivalent of 6 μg for 1 μg of RAE was used based on the presence of fat and the absence of a plant matrix (Institute of Medicine, 2001). Dietary Fe bioavailability was estimated by using the algorithms of Tseng et al. (1997) and Reddy et al. (2000) and adjusted for body iron stores (Cook et al., 1991). Percentage heme Fe in cooked animal products were estimated to be 60% for beef (Kongkachuichai et al., 2002; Lombardi-Boccia et al., 2002; Purchas et al., 2003; Turhan et al., 2004), 40% for pork (Kongkachuichai et al., 2002; Lombardi-Boccia et al., 2002), 30% for chicken (Kongkachuichai et al., 2002; Lombardi-Boccia et al., 2002; Turhan et al., 2004), 25% for fish (Kongkachuichai et al., 2002) and 10% for shrimps (Kongkachuichai et al., 2002). Estimated iron bioavailability was calculated for a mean meal size consumed by children 6-15y containing 28% sauce and 72% staple.

**Salt fortification**

Iodized salt from Namibia imported by the major Abidjan salt producer (Sagid Salt) was used for the study. This salt has a slight beige color, a range of a particle size from 0.2 - 2 mm, and a NaCl content of ≈ 97%. To produce the DFS, 300 g food-grade micronized FePP (art. no. 3046449, ≈ 25% Fe, mean particle size ≈ 2.5 μm, Dr. Paul Lohmann GmbH KG, Emmerthal, Germany), produced from regular FePP by grinding, was added to 25 kg of salt (3 mg Fe / g salt) and premixed by hand for several minutes with a plastic spoon. The premix was then fed in one pass through a 2 m long, screw ribbon blender set up by UNICEF at Sagid Salt for iodization.
Homogeneity tests showed a homogenous mixing of the Fe into the salt after one pass with no further improvement with additional passes; mean (SD) Fe concentration was 3.2 (0.2) mg Fe per g salt (n=30) after one pass through the mixer. A ≈30g sample from each mixing during the first 6 mo was placed into a tightly sealed plastic container, filled to minimize exposure to air and wrapped in aluminium foil to prevent exposure to light. It was then frozen at -25°C until Fe, iodine and color were measured. For the iodized salt, a ≈30g sample was taken out of the prepackaged 25 kg bags at Sagid Salt and similarly stored frozen until analysis for color and iodine.

The fortification level of 3 mg Fe/g salt was chosen on the basis of a mean per capita salt intake of 3.5 g/d in school children, an estimated iron bioavailability of ~14% from the diet (see analysis of the 3-day records in the Results section), a RBV of 70% of the micronized FePP compared to Fe sulfate in rats (Wegmuller et al., 2004), and the recommended level of daily Fe absorption (FAO/WHO, 1988).

**Stability testing**

DFS and IS were divided into 10 kg and 5 kg portions and stored in loosely-woven high-density polyethylene (HDPE) bags. This type of bag, woven from large flat strands of HDPE, is used to package salt at the production site. Two 300 g portions of DFS and IS were stored in transparent low-density polyethylene (LDPE) bags typically used at the retail level and in markets. The bags were stored in a clean, well-ventilated room out of direct sunlight, and evenly spaced on shelves to allow air circulation. The temperature and humidity were measured daily. The mean temperature and relative humidity (SD) during the 6 mo storage period were 27.5°C (2.1) and 79.3 % (9.9). At mixing and after storage for 1, 2, 3, 4, 5 and 6 months, ≈ 30 g aliquots of salt were collected in tightly-sealed plastic containers, completely filled to minimize exposure to air, wrapped in aluminum foil to prevent exposure to light, and then frozen until analysis for Fe (only the DFS), iodine and color. Each bag was thoroughly mixed before the aliquots were taken. For both salts, 3 aliquots were taken from the 10 kg bag, 2 aliquots from the 5 kg bag and 1 aliquot from each of the two 300 g bags.
Organoleptic testing

For the evaluation of sensory changes of local foods, IS and DFS were added to meals that were prepared by local women using traditional recipes. Two equal amounts of each of the common staples (rice, yam, cassava and plantain) were prepared in two pots and the same quantity of IS or DFS added. Four common sauces (tomato, eggplant, okra and palm nut) were prepared without salt, divided into two equal parts and equal amounts of IS or DFS added. The color, odor and flavor of these foods were compared by an untrained panel of 18-21 (per session) local adults (mean age 38 y; 20% female) using triangle tests (Meilgaard et al., 1991). In each session one staple and one sauce were evaluated. The panelists received 3 coded samples of the staple and 3 coded samples of the sauce. They were asked to start with the staple and to determine which sample differed from the other 2 samples and voluntarily describe how they differed (Meilgaard et al., 1991).

Efficacy study

The subjects were 5-15-y-old children from 4 primary schools. Informed written consent was obtained from the chief medical officer and informed oral consent from the school directors and the parents of the children. The Swiss Federal Institute of Technology in Zürich, Switzerland, and the Ministry of Health of Côte d'Ivoire gave ethical approval for the study. Oral assent was obtained from participating children. All children at the 4 schools (n=605) were screened. Height and weight were measured, a spot urine sample was collected for measurement of urinary iodine (UI) and blood was collected by venipuncture for the determination of hemoglobin (Hb), serum ferritin (SF), serum transferrin receptor (TfR) and C-reactive protein (CRP). Children with iron deficiency with or without anemia were invited to join the intervention trial. Two children with Hb <80 g/L were excluded and treated with oral Fe. The remaining children were divided into two groups: all children from two schools at one end of the village received the IS (Group 1; n=63); the children from two schools on the other end of the village received the DFS (Group 2; n=60). Both the investigators and schools were blind to group assignment. Each participating child was given a monthly 2.5 kg salt portion distributed at school to be used in their household. If a household had more than one child in the study, the salt was given to the oldest child. The 2.5 kg portion was based on a mean per capita salt intake of ≈ 4
g/d and an average household size of 12 persons. In a village meeting, it was emphasized that the distributed salt should be used for all cooking and food preparation, as well as at the table. This message was reinforced at each of the monthly salt distribution by local coworkers and the school directors. At the baseline screening and again at 4 months all children received an oral dose of 400 mg albendazol (BELTAPHARM SPA, Cusano Milanino, Italy & SmithKline Beecham Pharmaceuticals, Brentford, Middlesex,UK). At 6 mo, all baseline measures were repeated. To determine the prevalence of parasitic infections and micronutrient deficiencies that may influence response to the iron fortification, blood, spot urine and stool samples were taken from a subsample of ≈150 randomly selected children from all the screened children of the four schools for determination of parasitic infections (malaria (n=142), schistosomiasis (n=144), soil transmitted helminths (n=107)) 12 mo after the intervention, and in two other separate random subsamples (n=152 and 182), vitamin A and riboflavin status were determined at the 6 mo measures. At 18 mo, all baseline measures were repeated in the available children.

**Acceptability testing and compliance**

To judge DFS and IS acceptability, household interviews were conducted after 1 and 6 mo of salt use. The head of the household answered questions on patterns of salt use, acceptability and health benefits. Households were selected randomly: the first survey included 18 households in the IS group and 25 in the DFS group; the second included 50 households from each group. In total, 35% of participating households were surveyed. To estimate compliance, salt remaining in the household at the end of the month was weighed and the amount of salt consumed per day during the period since the last distribution calculated. This amount was divided by the number of people in the household and compared to the mean per capita salt intake from the 3-day weighed food records.

**Laboratory analysis**

Salt samples were stored at -25°C until analysis. Color was determined by colorimetry (Chroma-Meter CR-310, Minolta AG, Dietikon, Switzerland) as described in an earlier publication (Wegmuller et al., 2003) using the Hunter Scale. The color of
Efficacy of dual fortified salt

the DFS and IS at each time point was compared to IS at baseline and color lightness (L-value) and color difference (ΔE) was calculated.

For the determination of iodine content in the DFS, titration with sodium thiosulfate (WHO/UNICEF/ICCIDD, 1996) is complicated by the high concentration of Fe in DFS which interferes with the reaction of sodium thiosulfate and free iodine (Werlen, 2000). Therefore, salt iodine concentration was measured after salt samples were dissolved and diluted in distilled water using a modification of the Sandell-Kolthoff reaction originally described for the determination of urinary iodine (Pino et al., 1996; Haldimann et al., 2003; Wegmuller et al., 2003). This method for iodine determination in dual-fortified salt was compared to isotope abundance ratio measurements by ICP-MS and agreement between the two methods was excellent (r²=0.97). The pooled coefficient of variation (CVp) using the modified Sandell-Kolthoff method at 5 and 30 μg I/g salt was 9% and 6% (Haldimann et al., 2003).

The fortification level of Fe in the salt was verified by the standard addition method using flame atomic absorption spectroscopy (SpectrAA-400, Varian, Mulgrave, Australia).

Serum and urine samples were separated into aliquots and frozen at -25°C until analysis. Hemoglobin was measured by using an AcT8 Counter (Beckman Coulter, Krefeld, Germany) using 3-level controls provided by the manufacturer. SF was measured using an automated chemiluminescent immunoassay system (IMMULITE®, Diagnostic Products Corporation, Los Angeles, USA). TfR was measured using an enzyme-linked immunosorbent assay (RAMCO, Houston, USA). CRP was measured using nephelometry (TURBOX®, Orion Diagnostics, Espoo, Finland). Anemia was defined as Hb < 120 g/L in children aged ≥ 12 years, and Hb < 115 g/L in children aged 5 – 12 years (WHO/UNICEF/UNU, 2001). Iron deficiency was indicated by a TfR > 8.5 mg/L (Cook et al., 1992), or a SF < 30 μg/L (Van den Broek, 1998). CRP values < 10 mg/L were defined as normal (Pepys, 1981). Body iron stores were calculated from the TfR / SF ratio (Cook et al., 2003). Serum retinol (SR) was measured by HPLC (Merck-Hitachi, Tokyo, Japan) according to Tanumihardjo et al. (1994) with reference material from the National Institute of Standards and Technology (Gaithersburg, MD, USA); vitamin A deficiency was defined as a SR < 0.70 μmol/L (WHO, 1996). Riboflavin was measured by the EGRAC assay using a modification of the method of Dror et al. (1994). Riboflavin
deficiency was determined as values > 1.2 (Sauberlich, 1999). Urinary iodine (UI) was measured by using the Sandell-Kolthoff reaction as modified by Pino et al. (1996). Whole blood was used to prepare a thick and a thin smear for malaria parasites by the Giemsa coloration technique (WHO, 1990a). Parasites were counted against leukocytes; fields containing > 200 leukocytes were counted and if < 10 parasites were identified, the counting continued up to 500 leukocytes. These counts were converted to the number of parasites per µL of blood, assuming a leukocyte count of 8000/µL (WHO, 1990a). The presence of blood in urine, an indication for schistosomas, was analyzed by Dip Sticks (Roche Diagnostics GmbH, Mannheim, Germany). *Schistosoma haematobium* infection was measured in 10 mL of the spot urine sample, filtered with Nuclepore Filters (Sefar AG, Filtration Division, Heiden, Switzerland), using the syringe filtration technique. Filters were kept in Lugol solution (Merck, Darmstadt, Germany) until they were analyzed microscopically on the same day for the presence of *S. haematobium* eggs (WHO, 1990b). From the stool samples a thick smear was prepared using the Kato-Katz method (Katz et al., 1972). The slides were examined using light microscopy for the presence of eggs of soil transmitted helminthes (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*).

**Statistical analysis**

Data processing and statistical analysis were done using SPSS 12.0 (SPSS Inc., Chicago, USA) and Excel (XP 2002; Microsoft, Redmond, USA). Normally distributed data were expressed as means (SD) and were compared by Student’s *t* test. Parameters not normally distributed were expressed as medians and ranges, and were compared by Mann-Whitney and Wilcoxon tests or log transformed and compared by *t* test. A 2-factor ANOVA was done to compare effects of time and group and time-by-group interaction for Hb, indices of Fe status, salt iodine and salt color (lightness). *T* tests between groups and paired *t* tests within groups were done if the interaction effect was significant. The time effect for the binary variables of anemia, IDA and ID was tested by the McNemar test and the group effect by Pearson’s chi-square test. Significance was set at *P* < 0.05.
Results

Salt and micronutrient intake

The 3-day weighed food records were done in 24 families including 207 subjects (median age: 16 y; range: 2-81 y). Daily intakes of salt, iron, vitamins C and A, riboflavin, phytic acid and animal tissue (MFP: meat, fish and poultry) are shown in Table 1 and compared to the Estimated Average Requirement (EAR). Per capita salt intake was lower than previously reported for rural Côte d’Ivoire (Hess et al., 1999): in 2-5 y-old children, 6-15 y-old children and women, important target groups of iron and iodine fortification, mean daily salt intakes were 1.7, 3.4 and 4.5 g, respectively. Median vitamin A intake was at the EAR for 2-5 y-old children and above the EAR for 6-15 y-old children, adult females and males, due to high consumption of red palm oil and refined palm oil fortified with retinyl palmitate. Vitamin C intake was low (34-61% of the EAR) because consumption of fresh fruit was rare, and vegetables are consumed in sauces which are simmered for several hours resulting in extensive losses of vitamin C. Riboflavin intake was low (only 47-59% of the EAR) due to infrequent consumption of animal foods with the exception of fish, and the negligible riboflavin content of cassava, the dietary staple.
Table 1: Daily intake (median (range)) of salt, vitamin A (retinol activity equivalents (RAE)), riboflavin, ascorbic acid, iron, phytic acid and meat, fish or poultry (MFP) by 3-day weighed food records in Orbaff (n=207)

<table>
<thead>
<tr>
<th>Population group</th>
<th>Salt (g/d)</th>
<th>Iron (mg/d)</th>
<th>RAE (µg/d)</th>
<th>Riboflavin (mg/d)</th>
<th>Ascorbic acid (mg/d)</th>
<th>Phytic acid (mg/d)</th>
<th>MFP (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (2-5y)</td>
<td>1.3 (0-7.9)</td>
<td>5.5 (1.8-12.7)</td>
<td>247 (16-1733)</td>
<td>0.24 (0.09-0.53)</td>
<td>10.7 (3.6-63.6)</td>
<td>61 (46-848)</td>
<td>61 (7-167)</td>
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<td></td>
<td></td>
<td></td>
<td>153 (102)</td>
<td>53 (9)</td>
<td>153 (102)</td>
<td>53 (9)</td>
<td></td>
</tr>
<tr>
<td>Children (6-15y)</td>
<td>2.8 (0-10.9)</td>
<td>9.8 (3.6-19.8)</td>
<td>598 (68-2650)</td>
<td>0.42 (0.17-1.16)</td>
<td>20.9 (4.7-278.9)</td>
<td>199 (92-1233)</td>
<td>94 (45-335)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>156 (133)</td>
<td>47 (7)</td>
<td>156 (133)</td>
<td>47 (7)</td>
<td></td>
</tr>
<tr>
<td>Women (n=59)</td>
<td>3.8 (0-17.7)</td>
<td>13.4 (4.3-26.6)</td>
<td>822 (122-4363)</td>
<td>0.53 (0.20-1.13)</td>
<td>20.6 (2.8-181.0)</td>
<td>227 (82-1610)</td>
<td>124 (41-454)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>165 (164)</td>
<td>59 (9)</td>
<td>165 (164)</td>
<td>59 (9)</td>
<td></td>
</tr>
<tr>
<td>Men (n=45)</td>
<td>3.8 (0-11.2)</td>
<td>11.7 (2.0-23.2)</td>
<td>760 (17-5582)</td>
<td>0.54 (0.10-1.19)</td>
<td>30.8 (4.7-141.0)</td>
<td>230 (25-3005)</td>
<td>131 (43-263)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>195 (122)</td>
<td>49 (7)</td>
<td>195 (122)</td>
<td>49 (7)</td>
<td></td>
</tr>
</tbody>
</table>

1 EAR (Estimated Average Requirement) (Institute of Medicine, 2001) for iron is 3.6 mg for children (2-5y), 6.3 mg for children (6-15y), 8.1 mg for women and 6 mg for men
2 EAR for vitamin A (RAE) (Institute of Medicine, 2001) is 240 µg for children (2-5y), 450 µg for children (6-15y), 500 µg for women and 625 µg for men
3 EAR for riboflavin (Institute of Medicine, 1998) is 0.5 mg for children (2-5y), 0.9 mg for children (6-15y), 0.9 mg for women and 1.1 mg for men
4 EAR for vitamin C (Institute of Medicine, 2000) is 18 mg for children (2-5y), 49 mg for children (6-15y), 60 mg for women and 75 mg for men
Iron intake and bioavailability

Median iron intakes by age group are shown in Table 1. Median iron intake was well above the EAR for all age groups (Table 1). Ninety four percent of MFP intake was from fish, and 10% of dietary iron was heme iron, assuming fish to contain 25% heme Fe (Kongkachuichai et al., 2002). Phytic acid intakes were relatively low and in the range of 107-230 mg/d and mainly came from cassava, plantain and sauces with peanuts and okra. The molar ratio of phytic acid to iron in the diet was 1.4-1.7. Mean polyphenole intake was low in all age groups (55-157 mg/d). Mean total iron bioavailability (SD) in school age children assuming a SF concentration of 30 and 20 µg/L was 8.0 (2.2) and 11.4 (3.2)% for the Tseng model, and 11.9 (2.4) and 17.3 (3.4)% for the Reddy model, respectively (Tseng et al., 1997; Reddy et al., 2000). In school age children with a SF concentration of 20 µg/L, mean total daily iron absorption was estimated to be 1.16 and 1.76 mg for the Tseng and Reddy models, respectively, indicating adequate iron intake and bioavailability and that low Fe intake is not a major reason for ID.

Color and iodine stability

The color and iodine stability of the IS and the DFS are shown in Table 2. There was no significant difference of color lightness comparing the two salts and color lightness did not significantly change during storage. The ∆E of ≈ 11 at 6 mo was due to the slight difference in color between the DFS (light beige) and the IS (white) at baseline. Iodine loss at 6 mo was ≈ 50% for the IS and ≈ 70% for the DFS when stored in LDPE bags and close to 100% for both salts when stored in HDPE bags. Mean (SD) iron content of the DFS at mixing and after six months of storage was 3.28 (0.17) and 2.99 (0.24) mg Fe/g salt, respectively. There was no segregation in the dual fortified salt samples indicating that the small FePP particles adhere to the bigger salt particles.
### Table 2: Color and remaining iodine concentration in the iodized salt (IS) and the dual fortified salt (DFS) at mixing and after storage for 1, 2, 3, 4, 5 and 6 months\(^1\)

<table>
<thead>
<tr>
<th>Length of storage (mo)</th>
<th>Color(^2)</th>
<th>Iodine(^3,7)</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lightness(^4)</td>
<td>(\Delta E)(^5)</td>
<td>IS</td>
<td>DFS</td>
<td>IS</td>
</tr>
<tr>
<td>0</td>
<td>83.4 (0.7)</td>
<td>0.69 (0.44)</td>
<td>9.85 (0.92)(^6)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>78.8 (2.5)</td>
<td>4.55 (2.50)</td>
<td>9.43 (1.43)(^6)</td>
<td>14.2 (13.8)</td>
<td>34.6 (2.8)</td>
</tr>
<tr>
<td></td>
<td>50.2 (1.4)</td>
<td>48.0 (5.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>78.0 (2.9)</td>
<td>7.15 (4.05)</td>
<td>10.78 (2.15)</td>
<td>3.3 (2.5)</td>
<td>11.6 (3.8)</td>
</tr>
<tr>
<td></td>
<td>45.5 (2.9)</td>
<td>39.8 (6.0)</td>
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</tr>
<tr>
<td>3</td>
<td>78.0 (2.9)</td>
<td>5.39 (2.85)</td>
<td>11.11 (1.38)(^6)</td>
<td>1.2 (1.4)</td>
<td>11.4 (7.2)</td>
</tr>
<tr>
<td></td>
<td>48.3 (1.1)</td>
<td>38.8 (20.3)</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>79.1 (3.1)</td>
<td>4.81 (2.54)</td>
<td>12.46 (1.84)(^6)</td>
<td>3.2 (1.5)</td>
<td>16.8 (3.4)</td>
</tr>
<tr>
<td></td>
<td>54.0 (2.2)</td>
<td>40.9 (8.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>78.7 (3.2)</td>
<td>5.17 (2.65)</td>
<td>12.61 (1.66)(^6)</td>
<td>2.9 (1.0)</td>
<td>10.0 (8.0)</td>
</tr>
<tr>
<td></td>
<td>53.1 (0.2)</td>
<td>18.7 (0.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>75.3 (3.2)</td>
<td>8.04 (3.23)</td>
<td>10.98 (1.81)</td>
<td>2.1 (1.8)</td>
<td>7.6 (7.7)</td>
</tr>
<tr>
<td></td>
<td>47.0 (1.9)</td>
<td>33.0 (3.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)All values are means (SD)  
\(^2\)n=7 (5 samples from woven HDPE bags and 2 samples from transparent plastic LDPE bags)  
\(^3\)n=5 (samples from HDPE bags) in first line and n=2 (samples form LDPE bags) in second line  
\(^4\)Lightness scale: 0 = black, 100 = white  
\(^5\)\(\Delta E\), absolute color difference between DFS or IS and an IS reference sample  
\(^6\)Significant main effect of fortification, P < 0.05  
\(^7\)Significant main effect of time, P < 0.05
Organoleptic testing

In the triangle testing comparing DFS and IS, there was no detectable difference in color, odor or taste in either the traditional staples (rice, cassava, yam, plantain) or the sauces (tomato, eggplant, okra, palm nut).

Efficacy trial

The mean (SD) iodine concentrations of the monthly salt aliquots (14/mo and type of salt) taken during mixing of the salt during the first 6 mo were 71.5 (18.6) and 53.8 (18.2) μg/g salt in the IS and the DFS, respectively. The mean iron concentration in the DFS at the monthly mixings during the first 6 mo (3-7 aliquots/mo) was 3.21 ± 0.21 mg/g salt.

Screening

The results of the screening are shown in Table 3. Although nearly half of the children screened were anemic, the prevalence of IDA was only 12%, with another 11% of children iron deficient without anemia. Median UI concentration was 358 μg/L with a range from 19-1027 μg/L, indicating excessive iodine intake (WHO/UNICEF/ICCIDD, 2001). The mean (SD) SR concentration was 1.22 (0.38) μmol/L, and 7% of children had an SR < 0.7 μmol/L, indicating very little vitamin A deficiency. The mean (SD) EGRAC value was 1.26 (0.12), and 66% of children had a value > 1.2, indicating extensive riboflavin deficiency. Overall malaria prevalence was 55%; 50% and 5% of the children were infected with Plasmodium falciparum and P. malariae, respectively. However, parasite load was low (< 10'000 / μl blood) in 98% of the infected children. The prevalence of soil transmitted helminths was only 14%, probably due to deworming 9 mo prior to measurement. Only 11% of the screened children had a CRP > 10 mg/L.
Table 3: Results of the screening of all the children (n=605) in the four primary schools in Orbaff

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>9.2 ± 2.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.32 ± 0.15</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>27.8 ± 9.3</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>116 ± 12</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>66 (3-472)</td>
</tr>
<tr>
<td>Serum transferrin receptor (mg/L)</td>
<td>6.5 (2.3-29.7)</td>
</tr>
<tr>
<td>Prevalence of anemia [n (%)]</td>
<td>303 (50)</td>
</tr>
<tr>
<td>Prevalence of iron deficiency anemia [n (%)]</td>
<td>72 (12)</td>
</tr>
<tr>
<td>Prevalence of iron deficiency without anemia [n (%)]</td>
<td>64 (11)</td>
</tr>
<tr>
<td>Urinary iodine (µg/L)</td>
<td>358 (19-1027)</td>
</tr>
<tr>
<td>Serum retinol (µmol/L)</td>
<td>1.22 ± 0.38</td>
</tr>
<tr>
<td>Prevalence of vitamin A deficiency [n (%)]</td>
<td>10 (7)</td>
</tr>
<tr>
<td>Riboflavin EGRAC</td>
<td>1.26 ± 0.12</td>
</tr>
<tr>
<td>Prevalence of riboflavin deficiency [n (%)]</td>
<td>120 (66)</td>
</tr>
<tr>
<td>Prevalence of malaria [n (%)]</td>
<td>78 (55)</td>
</tr>
<tr>
<td>Prevalence of schistosomiasis [n (%)]</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Prevalence of soil transmitted helminths [n (%)]</td>
<td>15 (14)</td>
</tr>
</tbody>
</table>

1. Mean ± SD
2. Median (range)
3. Measured in a subsample of 152 children
4. Measured in a subsample of 182 children
5. Measured in a subsample of 142 children
6. Measured in a subsample of 144 children
7. Measured in a subsample of 107 children, 8 months after second deworming with 400 mg albendazole
6 months intervention

At baseline, comparing IS and DFS groups, mean (SD) age was 9.4 (2.9) and 9.5 (2.4), mean (SD) height was 1.32 (0.16) and 1.34 (0.14) and mean (SD) weight was 28.5 (9.5) and 29.2 (8.9), respectively, with no significant differences between groups. Groups were not significantly different for SF, TfR and body iron concentration at baseline but Hb was significantly higher in the DFS group than in the IS group due to the randomization at school level. Of the 136 children who were enrolled in the study, 13 children were absent from school at the 6 mo visit, so that 123 children completed the 6 mo measures. Changes in hemoglobin, SF and TfR concentrations and in prevalence of ID with and without anemia over the 6 mo intervention are shown in Table 4. There was no significant change in Hb in either group over the course of the study. Both SF and TfR concentrations significantly improved in the DFS group at 6 mo (P<0.05); there was no significant change in SF and TfR in the IS group. Figure 1 shows the significant increase in total body iron in the DFS group (P<0.001), with no significant change in the IS group. Mean (SD) body iron/kg body weight significantly increased in the DFS group from 4.6 (2.7) to 5.9 (2.7) mg/kg (P<0.001), whereas the change in the IS group from 5.5 (2.9) to 5.6 (3.1) mg/kg was not significant. However, at 6 mo only TfR was different between groups, but not SF and body iron. There were no differences in mean CRP or the prevalence of elevated CRP (11-15%) between the groups over the course of the study (data not shown). The prevalence of anemia did not significantly change between 0 and 6 mo with 62 and 62% in the IS and 42 and 47% in the DFS group. Both the prevalence of iron deficiency anemia (P<0.01) and iron deficiency without anemia (P<0.001) was significantly reduced in the DFS group. The prevalence of iron deficiency anemia, but not iron deficiency without anemia, was reduced in the IS group (P<0.02). Overall, the prevalence of iron deficiency with and without anemia was lower in the DFS group (51%) compared to IS (64%) at 6 mo, although this was not statistically significant.
Table 4: Hemoglobin, serum ferritin and transferrin receptor concentration and prevalence of iron deficiency with or without anemia of children in the iodized salt (IS, n=63) and dual fortified salt (DFS, n=60) groups at baseline and 6 months

<table>
<thead>
<tr>
<th>Time (mo)</th>
<th>Hemoglobin</th>
<th>Serum ferritin</th>
<th>Transferrin receptor</th>
<th>Iron deficiency with or without anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
<td>DFS</td>
<td>IS</td>
<td>DFS</td>
</tr>
<tr>
<td>0</td>
<td>112 ± 13</td>
<td>117 ± 13³</td>
<td>58 (10-472)</td>
<td>48 (9-397)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.1 (3.7-22.0)</td>
<td>9.2 (4.5-13.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58 (10-472)</td>
<td>48 (9-397)</td>
</tr>
<tr>
<td>6</td>
<td>113 ± 11</td>
<td>117 ± 12</td>
<td>61 (11-500)</td>
<td>63 (6-200)¹⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.2 (4.7-16.6)</td>
<td>8.2 (3.0-16.1)¹⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>61 (11-500)</td>
<td>63 (6-200)¹⁶</td>
</tr>
</tbody>
</table>

¹ Significant treatment x time interaction for all variables, P< 0.001 (ANOVA)
² Mean ± SD
³ Median (range)
⁴ For serum ferritin children with CRP values > 10 mg/L were excluded (IS, n=49; DFS, n=46)
⁵,⁶ Significantly different from baseline: ⁵P<0.01, ⁶P<0.05
⁷ Significantly different from the IS group: ⁷P<0.05
18 months intervention

As mentioned above, the 12 mo measures were not possible to do due to the political situation in Côte d'Ivoire which did not allow traveling in the country. Salt distribution was therefore continued until 18 mo although distribution was interrupted or delayed some times. Many children enrolled in the study were not available for this time point measurement because they moved away or they were from the highest class of the primary school and therefore had moved to secondary school in town. The final number of children who completed the 18 mo intervention were 35 in the IS and 34 in the DFS group. Nevertheless, statistical analysis were performed (Table 5) and showed a significant difference in Tfr after 18 mo (P<0.05) but not after 6 mo in the DFS group and no change in the IS group. There was no difference in Tfr between groups at any of the time points. Total body iron improved significantly after 18 mo (P<0.01) but not after 6 mo in both groups. Hb and SF did not change over time in either of the two groups. Iron deficiency without anemia was significantly reduced in the DFS group after 6 and 18 mo (P<0.01) whereas no change was observed in the IS group. However, one needs to consider that ID prevalence was significantly higher in the DFS group at baseline compared to the IS group (P<0.05). In contrast, IDA
prevalence was significantly higher in the IS group compared to the DFS group (P<0.05) and significant reduction at 6 and 18 mo only occurred in the IS group (P<0.01) but not in the DFS group. Anemia prevalence as well was significantly different between groups at baseline (P<0.05) with a higher prevalence in the IS group and a significant reduction at 18 mo in this group (P<0.05) but with no change in the DFS group over time. Overall, the prevalence of iron deficiency with and without anemia was significantly reduced at 6 and 18 mo (P<0.01) compared to baseline in both groups and no difference between groups at any time point was detected. There was an increase of median urinary iodine concentration from 394 to 474 μg/L in the IS and from 360 to 482 μg/L in the DFS group after 6 mo, indicating the consumption of highly iodized salt. However, at 18 mo the median UI concentration decreased to 222 and 240 μg/L in the IS and DFS group, respectively.
Table 5: Hemoglobin, serum ferritin, transferrin receptor and body iron concentration and prevalence of iron deficiency with or without anemia of children in the iodized salt (IS, n=35) and dual fortified salt (DFS, n=34) groups at baseline and 6 and 18 months

<table>
<thead>
<tr>
<th>Time (mo)</th>
<th>Hemoglobin¹</th>
<th>Serum ferritin²,³</th>
<th>Transferrin receptor²</th>
<th>Body iron¹,³</th>
<th>Iron deficiency with or without anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS IS DFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>113±11 117±12</td>
<td>96 (23-274) 66 (10-231)</td>
<td>9.0 (4.1-13.8) 9.1 (4.5-13.9)</td>
<td>6.2 ± 2.1 5.3 ± 2.6</td>
<td>100 100</td>
</tr>
<tr>
<td>6</td>
<td>115±10 117±12</td>
<td>67 (25-392) 72 (18-166)</td>
<td>8.6 (4.7-13.1) 8.1 (4.1-16.1)</td>
<td>6.3 ± 2.3 6.1 ± 2.4</td>
<td>54⁴ 50⁴</td>
</tr>
<tr>
<td>18</td>
<td>115±14 120±13</td>
<td>88 (46-525) 89 (26-246)</td>
<td>8.2 (4.3-12.1) 8.3 (5.1-18.5)⁵</td>
<td>7.6 ± 2.4⁵ 6.9 ± 1.8⁴</td>
<td>40⁴ 44⁴</td>
</tr>
</tbody>
</table>

¹Mean ± SD
²Median (range)
³For serum ferritin and body iron children with CRP values > 10 mg/L were excluded (IS, n=22; DFS, n=17)
⁴,⁵Significantly different from baseline: ⁴P<0.01, ⁵P<0.05
**Household acceptability and compliance**

Results of the acceptability surveys after 1 month and 6 months of salt use in the households are shown in Table 6. Most households reported the 2.5 kg monthly distribution was adequate to cover all household needs. Salt was used to prepare sauces and the main staples in 92 -100% of the households. All surveyed households rated both IS and DFS as acceptable. Although 52% and 36% of DFS households during the first and the second survey, respectively, stated that addition of the DFS darkened food, no perceptible darkening of sauces or tubers was detected in the systematic triangle testing of the DFS. To estimate compliance, salt remaining in the household at the end of the month was weighed, and the amount of salt used was compared to the expected per capita salt consumption from the weighed food records. Mean (SD) per capita salt intake from the weighed food records was 3.7 (3.1) g/d. Calculated per capita salt consumption in the households using the DFS was 4.0 (2.5) and 6.1 (4.0) g/d at month 1 and 6, respectively, suggesting compliance with the DFS was high for the first 6 mo. However, no such measures were repeated after 6 mo due to the political situation.
Table 6: Acceptability of iodized salt (IS) and dual fortified salt (DFS) after 1 and 6 months of salt use in the households

<table>
<thead>
<tr>
<th>Question</th>
<th>Salt</th>
<th>1. survey</th>
<th>2. survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
<td>DFS</td>
<td>IS</td>
</tr>
<tr>
<td>1. What amount of salt used 3 wk (1. survey) or 2 wk (2. survey) after distribution</td>
<td></td>
<td></td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>2. Was salt used for a selection of foods?</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>3. Was the salt different or did the addition of the salt change the food?</td>
<td>No change</td>
<td>82</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Turned the food black</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Salt sweeter than normally consumed salt</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Saltiness of salt different from normally consumed salt</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Change of taste of the food</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4. Was the salt different and was it acceptable</td>
<td>Not different</td>
<td>82</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Different but acceptable</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Different, not acceptable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5. Did you observe any change concerning the health of the family?</td>
<td>No change</td>
<td>74</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Negative change</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Positive change</td>
<td>22</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Question 5 was only asked during the 2nd survey
Discussion

Dual fortification of salt with iron and iodine is technically challenging. Its success depends on the chemical form of the iron and iodine compound, on salt quality, on storage conditions and packaging material (Venkatesh Mannar & Diosady, 1998; Wegmuller et al., 2003). There have been several previous attempts to use ferrous iron in a DFS. A DFS containing ferrous fumarate and KI coated with maltodextrin was developed (Diosady et al., 2002), but the ferrous fumarate caused unacceptable reddish color changes during storage in African salt (Wegmuller et al., 2003). A DFS containing ferrous sulfate encapsulated with hydrogenated soybean oil and KI was highly effective in reducing the prevalence of IDA in Moroccan schoolchildren (Zimmermann et al., 2003b). However, it produced a yellow color change in salt during the damp season when the moisture content of the salt was 3-4%. When added to local salt in North and West Africa, currently-available commercial encapsulated ferrous iron compounds precipitate unacceptable color changes (Wegmuller et al., 2003). This may be due to moisture penetration through plant oil-based capsules or abrasion of the capsules during mixing. Additional limitations to using oil-based capsules are their low melting point and the possibility that Fe is release during food preparation to provoke sensory changes (Hurrell, 1985), and their relatively high costs (Zimmermann, 2004a).

FePP is a white colored, insoluble and organoleptically inert iron compound. It can be fortified into even low-grade salt without precipitating color changes (Wegmuller et al., 2003). Moreover, ferric iron is more stable than ferrous iron when used in a DFS together with iodine in the form of KIO₃, the form of iodine recommended for salt fortification in developing countries (WHO/UNICEF/ICCIDD, 2001). However, the lower relative bioavailability of FePP compared to ferrous sulfate has presumably limited its use in food fortification (Hurrell, 2002b; Wegmuller et al., 2004). However, Zimmermann et al. (2004b) recently showed that increasing the fortification level of an iron compound can compensate for its lower bioavailability. Regular FePP (MPS ≥ 10 pm) has been reported to be 20 – 70% the RBV of ferrous sulfate (Hurrell, 2002b; Wegmuller et al., 2004). Reducing the particle size to 0.5 pm has been reported to increase RBV of FePP in milk products (Fidler et al., 2004c). However, we have no evidence that the micronized ground FePP (MPS = 2.5 μm) has a higher
bioavailability than the regular FePP. In the present study, based on the change in body iron after 6 mo of DFS, considering the mean salt intake in school children of 3.4 g/d and the fortification level of 3 mg Fe/g salt, approximately 3 - 3.5% of the fortification iron was absorbed during the study period.

A significant advantage to the approach to salt fortification in this study was the achievement of a homogenous mixture of the iron into the salt with one pass through the local ribbon screw mixer already in place for salt iodization. Similar screw mixers have been installed in many countries by UNICEF to support salt iodization, and their use to produce a DFS would mean the only additional costs would be the iron compound and installation of a dry-mixing device to dose the iron into the upper basket of the screw mixer.

Results of the 18 mo intervention are very inconsistent most probably due to irregular salt consumption during the vacation time of 4 mo. Although salt had been picked up from most of the children during vacation time, it is very likely that children spent some time in other villages or in the capital Abidjan, or that they ate in other families in the same village, who did use the other type of salt. In addition, there was 1 mo without any distribution at all and several delays in other months due to the unstable political situation. The decreasing UI concentration also is an indication that children did not consume the study salt which was always iodized at a very high level. In addition, sample size was quite small with 35 and 34 children in the IS and DFS group, respectively, and was even smaller after elimination of children with a CRP > 10 mg/L which was used for SF concentration and the calculation of body iron (IS, n=22; DFS, n=17). For all this reasons and uncertainties which are reflected in the inconsistency of the results presented in Table 5, the discussion about the efficacy of DFS is limited to the 6 mo intervention.

This study was similar to a previous study conducted in Morocco in which a DFS containing micronized FePP decreased the prevalence of IDA in children from 31% to 3% after 10 mo (Zimmermann et al., 2004b). There are several possible explanations for the blunted response seen in Côte d'Ivoire. The present study length was 6 mo compared to 10 mo in Morocco, and the daily iron dose given here was ca. 10 mg/d, compared to ca. 20 mg/d in Morocco. Another difference between the 2
study sites was the food vehicle. While in Morocco salt was mainly baked into bread, it was almost completely added to sauces in Côte d'Ivoire, so some FePP may have been lost in the cooking pot as it is insoluble. A third difference was the relatively low level of ID in Côte d'Ivoire in the region where this study was done due to a relatively high iron intake and bioavailability due to regular fish consumption. Although there was a high prevalence of anemia in the Ivorian school children (50%) only 12% had IDA. Along with iron deficiency, anemia in children in developing countries may be due to concurrent micronutrient deficiencies, malaria, hookworm, and hemoglobinopathies (Ramakrishnan, 2001; Thurlow et al., 2005). Malaria is endemic in Côte d'Ivoire (Staubli Asobayire et al., 2001; Raso et al., 2004) whereas there is no malaria in northern Morocco. Although the malaria prevalence was determined in the dry season, during which malaria is less common, 55% of children had low-level parasitemia. Studies of DFS efficacy in India also reported no effect on Hb, possibly due to a high prevalence of malaria in the study population (Sivakumar et al., 2001). Because of parasites in Côte d'Ivoire, we treated with albendazole at baseline and 4 mo. This could explain the impressive decrease in IDA in the control IS group. Hookworm and amoebae infection are common in rural Côte d'Ivoire; with studies reporting infection rates of 35-45% and 42% of hookworm and Entamoeba histolytica/E. dispar (Staubli-Asobayire, 2000; Raso et al., 2004). Blood loss due to hookworm infection can contribute to iron deficiency and anemia (Brooker et al., 2004) and several studies have shown a strong association between intensity of infection and anemia (Stoltzfus et al., 1997; Stoltzfus et al., 2000).

Another comorbidity that may have blunted the response to iron fortification was the poor riboflavin status in Ivorian children. Riboflavin deficiency may produce alterations in iron metabolism and Hb synthesis (Ramakrishnan, 2001). It may impair the synthesis of globin, and reduce the activity of NADH-FMN oxidoreductase so that iron becomes trapped in ferritin (Ramakrishnan, 2001). In a trial with riboflavin deficient individuals supplementation with the vitamin caused an increase in Hb concentrations and an improved hematological response to iron supplements in iron deficient Gambian men and lactating women (Powers et al., 1983b; Powers et al., 1985a). The high prevalence of riboflavin deficiency in children in Côte d'Ivoire may have contributed to the lack of Hb response despite an increase in body iron.
A similar pattern of response has been reported in previous Fe repletion trials in tropical countries. In preschool children in Zanzibar, iron supplementation had no effect on Hb concentration or mild or moderate anemia but improved SF and erythrocyte protoporphyrin (Stoltzfus et al., 2004). The authors suggested the lack of effect on Hb was due to endemic infections and concurrent nutrient deficiencies.

Our findings indicate a DFS containing micronized FePP can increase body iron stores in children in Côte d’Ivoire, but not increase hemoglobin. These data suggest that iron fortification programs may not be successful in reducing anemia in tropical West Africa without concurrent control of endemic parasitoses.

Acknowledgements

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Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L & Tielsch JM (2000) Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. J Nutr 130, 1724-1733.


Conclusions and Perspectives

CONCLUSIONS AND PERSPECTIVES

Dual fortification of salt with iodine and iron has been confirmed to be challenging in the first study of this thesis. The testing of a wide range of commercially available iron compounds, including encapsulated iron compounds, showed that only few compounds are feasible for fortification of low grade salt in North and West Africa. In particular, encapsulated compounds were found to be of little use for salt fortification most probably due to mechanical abrasion during mixing and/or leaking of iron from the capsules. With the exception of a wax coated fumarate compound (with no knowledge of its bioavailability), color change and iodine loss were not greatly reduced compared to salt fortified with nonencapsulated compounds suggesting that currently available encapsulation techniques for iron need to be improved. The difficulty of using encapsulated compounds for salt fortification was confirmed by a study by Zimmermann et. al. (Zimmermann et al., 2003b) which showed that salt dual fortified with iodine and encapsulated ferrous sulfate underwent color changes during the damp season in Morocco. Salt fortified with nonencapsulated ferrous sulfate was stable when SHMP, a stabilizing agent, was added. However, the addition of stabilizers is not desirable since this complicates the mixing of the salt due to an additional ingredient and at the same time additional costs for the additives are generated. The most promising iron compound turned out to be ferric pyrophosphate due to its white color and its inert sensory properties. However, due to the relatively low bioavailability of 20-70% (Hurrell, 2002b) compared to ferrous sulfate this compound was for a long time neglected, but seems likely to be the only option to fortify white food vehicles such as rice and salt.

Expecting many people in the developing world to suffer from more than one micronutrient deficiency, microcapsules using palm fat as the coating material containing iron, iodine and vitamin A were produced by spray cooling in order to prevent iodine loss due to reactions with salt impurities and moisture as well as to improve the effect of each single micronutrient which have been shown to interact with each other (Hess & Zimmermann, 2004). By using ferric pyrophosphate color stability was acceptable and stability of iodine and iron when microcapsules were added to local Moroccan salt during storage were good. However, iodine and vitamin A losses during production of the microcapsules were relatively high indicating that
further optimization of the spraying process are needed. A study with school children consuming salt fortified with the microcapsules showed high efficacy of all 3 micronutrients in Morocco (Zimmermann et al., 2004a). All these findings indicate that salt fortified with a single capsule containing the 3 most important micronutrients could be a useful and simple new fortification strategy in countries where people live from subsistence farming and salt is likely to be one of very few purchased foods. However, production costs need to be reduced if used for fortification programs in developing countries.

Since ferric pyrophosphate was found to be almost the only iron compound feasible for salt fortification the question arose whether its low bioavailability could be improved by particle size reduction. In a rat study comparing the bioavailability of regular FePP (MPS = 21 μm) and two micronized FePP (MPS = 2.5 μm and 0.5 μm) it was found that the 0.5 μm compound had significantly higher bioavailability than the other two compounds and comparable bioavailability to ferrous sulfate. However, this compound is patented and therefore prohibitively expensive and its iron content is rather low and therefore big amounts would have to be added to the food. The 2.5 μm compound showed higher bioavailability than the regular compound, but the difference was not statistically significant and therefore can not be recommended to be preferred to regular FePP, which is less expensive. The effect of encapsulation was also evaluated in the same study and showed that encapsulation decreased bioavailability of the 2.5 μm compound significantly indicating that the capsule:substrate ratio of 60:40 needs to be decreased.

Finally, the organoleptic acceptability and efficacy of local salt dual fortified with the 2.5 μm FePP and KIO₃ was tested in Côte d’Ivoire. Sensory tests showed no differences in meals prepared with DFS or IS. An important finding was that DFS can be produced with one pass through the ribbon screw mixer already in place for salt iodization. The efficacy study showed an improvement in iron status but not in hemoglobin in children consuming DFS whereas no change was observed in children consuming IS after 6 months. These findings of a blunted efficacy were rather surprising when compared to a study conducted in Moroccan school children using the same DFS (Zimmermann et al., 2004b) and showing high efficacy in reducing the IDA prevalence. There are several reasons for the blunted effect in Côte d’Ivoire: First, the study site is climatically different with a tropical climate in Côte d’Ivoire resulting in a high malaria and infection prevalence. These factors are known to
Influence iron metabolism through blood loss, increased hemolysis of red blood cells and decreased absorption (Ramakrishnan, 2001). Second, several other micronutrients interact with iron in the metabolism and thereby can increase the anemia prevalence. The prevalence of riboflavin deficiency was found to be 66%, that of malaria 55% and that of soil transmitted helminths 14% in the study population. All these factors probably were responsible for the high prevalence of anemia (50%) of which only 1/5 was identified as IDA. Although only children with ID or IDA were included in the study the above mentioned factors most probably had a significant influence on the effect of the iron absorption and utilization from DFS. Third, the ingested amount of iron was rather low due to low salt consumption in Côte d'Ivoire and the study duration of 6 months was short. In conclusion, although DFS was highly efficacious in Morocco it may not be successful in reducing anemia prevalence in tropical countries without concurrent control of endemic parasitoses.

Overall, the results of this thesis showed that stable DFS can be produced by fortification with FePP and KIO3 by using the mixer for salt iodization already in place in many developing countries. The new approach of using a compound with a low bioavailability but adding higher amounts showed promising results and is likely to be the only way to fortify difficult to fortify food vehicles such as rice and salt. However, the question remains, whether particle size reduction of FePP to 2.5 μm is reasonable and bioavailability needs to be compared directly to regular FePP. Probably most important, to treat and prevent iron deficiency in tropical countries iron fortification programs need to be combined with other health interventions in order to succeed.

References
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