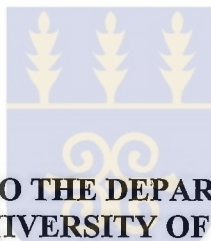


**THE EFFICACY OF DOUBLE-FORTIFIED SALT IN
REDUCING IODINE DEFICIENCY AND ANEMIA**

BY

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DECLARATION

This project was conducted and presented by me as part of the Micronutrient Initiative (MI) sponsored project at the Department of Nutrition and Food Science, University of Ghana, legon, under the supervision of Dr. Ebenezer Asibey-Berko.



.....

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DEDICATION

This work is dedicated to all underprivileged children and those fighting for their better future.



ABSTRACT

About 1.6 billion people in more than one hundred countries including Ghana suffer the consequences of micronutrient deficiencies, namely iron, iodine and Vitamin A. A thousand million people were estimated to be at risk of iodine deficiency alone in 1990. Two hundred million have goitres, 20 million are mentally retarded; six million of these being cretins. Iron deficiency anemia is the most widespread nutritional deficiency in the world today. Two billion people are affected by anemia, more than half being preventable and treatable anemia. Prevalence rates are higher in developing countries. The most affected are women of child-bearing age, and pre-school children (six months to five years of age). The purpose of this study were to assess the efficacy of the double fortified salt in preventing anemia and iodine deficiency disorders. A total of 289 children aged between 1- 5 years old whose parents gave their consent and also qualified to participate in the study were initially screened at baseline for hemoglobin. Out of this, a total of 150 children had their hemoglobin $\geq 10\text{g/dL}$. The selected children were assigned to one of the three intervention salt groups in a randomized controlled double-blind design for four months. One group was assigned to the red-labelled salt (R) suspected, by virtue of its color, to be double fortified and the other two groups on green- labelled salt (G) and

yellow-labelled salt (Y) -both believed to be iodized salt. Salt was supplied constantly to ensure that the study subjects do not run short. They were also asked not to buy or use any salt from the market. They were monitored weekly to ensure compliance of the usage of the salt as well as its acceptability. Urine and blood samples were collected at the baseline and the fourth month to estimate the amount of iodine and iron nutrition status of the children. Malaria parasite, sickle cell and serum ferritin were also determined. Seventy-one (71) percent of the children screened at the baseline were found to be anemic ($Hb < 11g/dL$) and 80% found to be iodine deficient (i.e $UI < 10\mu g/dL$). Mild anemia was found to decrease by 27% for children on the double fortified salt. Mild anemia (i.e $Hb \geq 10g/dL$ and $< 11g/dL$) among the children on the 'Y' and 'G' salts were also found to decrease by 20% and 8% respectively. However, there was no significant difference ($p = 0.4$). between the mean hemoglobin of the children on the three different salts. Prevalence of malaria was found to be low (5.5%), probably due to the time the research was conducted (dry season). Twenty-one per cent of the children were found to have sickle cell anemia but this could not be the cause of the high rate of anemia found among the children. This is because, 50% of those with sickle trait had their hemoglobin higher than 10g/dL. The median ferritin concentration of the children on the double

fortified salt was found to have performed better (increased by $25.8\mu\text{g/L}$ at the end of the 4-month period) than that of those on the other two salts (Y=17.8 and G=22.3) but there was no statistical difference ($p = 0.2$) between the salt groups. The rate of iodine deficiency found among the children at the baseline was 71.4%. Out of this, 29.6% were found to have mild deficiency, 17.4% had moderate deficiency and 24.6% also had severe iodine deficiency. The iodine status of the children improved at the end of the study. Only 20.3% were still found iodine deficient. This may be due to the very low urine iodine levels ($0.09\mu\text{g/dL}$) of some of the children at the baseline. Hence the period of study (4th months) may not be enough for such children to catch-up. Again, those on the double fortified salt was found to have performed better than the other two salts. Those on the double fortified salt had an improvement of 67%, those on the Y-labelled salt was also improved by 39.4% whereas those on the G-labelled salt was increased by 41%. The double fortified salt could therefore be said to be effective or efficacious in alleviating iodine deficiency.

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CHAPTER ONE

INTRODUCTION AND OBJECTIVES

INTRODUCTION

1.1 FOOD FORTIFICATION

Food fortification is defined as the addition of nutrient(s) to food in order to maintain or improve the nutritional quality of individual foods or the total diet of a group, community, or a population (Finch and Monsen, 1972). Food fortification may offer considerable nutritional benefits under certain circumstances. It may be the most feasible approach to certain nutritional problems. Two specific examples are the iodization of salt and bread. In some circumstances, food fortification may complement other intervention activities in a synergistic manner. When new foods are being introduced into a community it may be possible to modify their nutritive values through fortification to obtain maximum effect. A special type of fortification is involved in the preparation of various formulations such as weaning foods, in which the total nutrient content is important and requires to be adjusted through the inclusion of selected additives. In the industrialized countries,

fortification finds increasing application in compensating for the effects of processing and refining on the nutritive values of foods. While this problem may reach greater proportions in countries with highly developed food industries, the relation between beriberi and polished rice for example, shows that (cereals) with a reduced nutrient content are marketed in most, if not all of the developing countries. Food fortification is likely to prove most beneficial when one or more nutrients is in short supply in the community, especially in a segment of the population, but when the total amount of food available is not seriously inadequate. Fortification improves the quality of food supply without affecting the quantity of food.

Double fortification of salt with iron and iodine is a classical example of a well-designed program, which will provide obvious benefits. However For more than twenty years, scientists have tried to fortify salt with both iron and iodine in a way as to prevent them from reacting and losing their potency (Boutet, 1997). This is because when iron and iodine are used to fortify salt, the iron is oxidized to the ferric state, becomes less available and has an unpleasant taste and unattractive yellowish brown color. The iodine (iodide or iodate) is also converted to elemental iodine, which sublimates and is lost rapidly. It has been reported that at a temperature of 40°C and a relative humidity of

100%, the highest losses of iodine occur (Diosady, 1996). Magnesium Chloride, which is a hygroscopic impurity of salt, increases the moisture content, causing almost immediate loss of over 90% of iodine in salt. Adequately refined salt may help to resolve this problem by eliminating Magnesium Chloride. Also absorbed water may be prevented from coming into contact with both iron and iodine on the salt surface, (Diosady, 1996).

1.2 DOUBLE FORTIFICATION OF SALT - A BREAKTHROUGH

As already mentioned when iron and iodine interact, the amounts of the two nutrients available for absorption by the human body is significantly reduced.

(Diosady, 1996). However, the National Institute of Nutrition, India has reported the production of double-fortified salt with iron and iodine which is stable for about seven months (Bagepallis and Navasinga, 1994). An earlier report also indicated the marketing of this salt in Tamil Nadu (SCN, 1993). However, research based on double fortification must continue in order to optimize the stability of iodine and iron in the product.

A recent breakthrough in the food-fortification technology that allows these two essential nutrients –iron and iodine – to be added to salt represent a major advance in international efforts to prevent health problems associated with micro-nutrients deficiencies. This breakthrough involves encapsulating

the iodine particles with a layer of dextrin, a water soluble starch compound, which serves as a physical barrier between the two elements. This technology was developed with the support of the Micronutrient Initiative (MI) and the International Development Research Center (IDRC)

Iodine fortified salt was introduced in North America and parts of Europe during the 1920s and has recently been introduced into Ghana. A law for the use of iodated salt came into an effect in Ghana in December, 1997, (Daily Graphic, 1996). However, today, a lot of the populace is still using uniodated salt, especially in the rural areas.

In theory, iron and iodine fortified salt can simultaneously combat or eliminate iron and iodine deficiency disorders, which together affect one-third of the world's population.

1.3 AVAILABILITY OF IRON AND IODINE IN THE DOUBLE FORTIFIED SALT

The availability of iron and iodine from the salt was investigated by Zlotkin *et al* in 1995. The dextrin-coated iodine was found to be well absorbed and is readily available from the salt in meals. The study also demonstrated the acceptable levels of absorption of the iron, in the form of ferrous fumarate,

from the double fortified salt in meals (Masoud and Zlotkin 1995). Factors such as vitamin C, protein, cysteine and all other factors, which influence the absorption of non-hem iron from food, influence the absorption of iron in the salt. It is also inhibited by compounds like phytates, polyphenols fiber and tannins.

The purpose of this study were, to test the acceptability of the double fortified salt and to verify if the salt may impart any unpleasant taste and color to any of the commonly consumed Ghanaian dishes and secondly, to investigate its efficacy to determine whether the salt could prevent anemia and iodine deficiency disorders.

1.4 SENSORY EVALUATION

The quality of a product may be easily determined by the consumer. The appearance could be determined by the eye, aroma by the sense of smell and texture by physical touch and feeling.

To specifically test these food qualities, there are three major types of sensory evaluations or tests. Preference or acceptability test, discriminatory test and descriptive test. In the preference or acceptability test, the personal attitude or feeling of the panelist towards a product directs his response. Discriminatory test is used to determine whether any difference exists between samples as

well as the nature and intensity of the difference (Ihekoronye and Ngodi, 1985). The panelist does not allow his personal likes and dislikes to influence his response.

When there are more than two samples to be evaluated, each must be compared with every other sample. The number of pairs compared in such a case is determined by the formula $0.5n(n-1)$ where n is the number of samples. This test is called the multiple paired comparison tests. In multiple comparison tests, the samples being tested are first analyzed for any selected characteristics, using analysis of variance. Where a significant difference is observed, a multiple range tests is applied to all possible pairs of samples in order to identify which pairs differ significantly.

In multiple paired comparison test, judges or panelists are presented with a known or reference sample R together with other test samples. These test samples must be coded. The panelists are asked to compare the coded samples with the reference sample R on the basis of some known characteristics, example, taste, appearance and overall acceptability. This procedure may be used effectively to compare four or more samples.

1.5 STATEMENT OF THE PROBLEM

Iron and Iodine deficiency disorders together affect one-third of the world's population. Iron deficiency is the most common nutritional problem in the world, particularly among women of childbearing age and children, (Ending Hidden Hunger, 1991/ACC/SCN, 1992/ FAO/WHO/ICN, 1992). Two billion people are affected by anemia, more than half being preventable and treatable anemia. Prevalence rates are higher in developing countries. Iron deficiency, if uncontrolled, will lead to increasingly severe anemia, reduced work capacity, reduced learning ability due to impaired cognitive development, increasing susceptibility to infections and a greater risk of complications or death of mothers during childbirth. About 50% of women of childbearing age in the world are estimated anemic as a result of iron deficiency. Africa is leading with 60% expectant mothers and children (0-5 years) affected. (FAO/WHO/ICN, 1992).

In Ghana the inadequacy of dietary iron intake appear to exist around the country, especially among the rural and urban poor. Twenty three percent children admitted to Axim hospital in 1991 had severe (Hb < 5g/dl). Ninety five percent of these were pre-scholars (Addo, 1991 Nutrition Division Ministry of Health, Ghana). By WHO (1968), standards (Hb<11.0g/dl),

63.7% pregnant hospital attendants were found anemic in 1973 (n=1098), 69% of pregnant women were found anemic at antenatal clinics in 1987. In 1991, the Blood Transfusion services in Ghana found 40% of all its blood transfusion going to children because of high rates of anemia among them (Nutrition Division, MOH). Anemia in Ghana is further aggravated by malaria, intestinal worm infestation and other infections.

Iodine deficiency also causes mental and physical retardation, goiter, and spontaneous abortion. One thousand million people were estimated to be at risk of iodine deficiency alone in 1990. Two hundred million have goiters, 20 million are mentally retarded –six million of these being cretins, (PAMM 1992 Country Program, Ending Hidden Hunger, 1991, ACC/SCN, 1992). Iodine is a public health problem in twenty seven African countries with 227 million at risk in Africa alone, (ACC/SCC, 1992, FAO/WHO/ICN 1992). In Ghana the National IDD survey found at least 33 % of the country's 110 districts affected by severe IDD (Asibey-Berko and Orraca-Tetteh, 1994). IDD is the leading cause of intellectual and neurological impairment in the world. Iodine deficiency leads to brain damage in the fetus, causing irreversible neurological damage to the brain. Its effect on the reproductive functions are increased rates of abortion, stillbirth, congenital abnormalities, low birth weights and infant mortality rates, (Ending Hunger, 1991).

Much of the retardation can be averted by correction of the deficiency during pregnancy, but if it goes uncorrected, the child's intelligent quotient (IQ) may get as low as 20%, (Hamilton *et Al.* 1988.) Iodine deficiency may either result from inadequate dietary intake or presence of goitrogenous substances in the food. The goitrogens present in the food may inhibit the iodine in the food from being absorbed in the gut, resulting in deficiency.

Some drawbacks to any program based on medication with iron supplements should be noted. First, in the case of injections, the treatment is somewhat painful and can leave permanent scars on the skin at injection sites. These factors might well reduce community acceptance of any injection scheme. Pills also have certain disadvantages. There may be a high rate of intolerance upsets. These problems could cause a decreased compliance with intake.

The manufacture and distribution of salt fortified with iron and iodine has been suggested to be inexpensive and an effective means of preventing iron and iodine deficiency disorders. Before the recommendation of the double fortified salt, the next important step was the determination of the efficacy of the double fortified salt in preventing iron deficiency anemia and alleviation of iodine deficiency.

It is suggested that the elimination of iron deficiency alone can do more than any single program to achieve the goals of development because both

sexes of all ages are affected.

1.6 OBJECTIVES

1.6.1 GENERAL OBJECTIVES

1. To investigate the acceptability of double fortified salt in Ghana
2. To investigate the efficacy of double fortified salt in controlling iron deficiency anemia and iodine deficiency disorders among children of 1-5 years old in Ghana

1.6.2 SPECIFIC OBJECTIVES

1. To determine the effect of double fortified salt on some common Ghanaian dishes.
2. To determine the overall acceptability of double fortified salt.
3. To investigate the prevalence of iron deficiency anemia and iodine deficiency among children of 1-5 years old in the Sekyere west district of Ghana.
4. To investigate if iron/iodine fortified salt maintains iron status and improves iodine status of children in the study area.
5. To compare iodine status of children on double fortified salt with those on iodized salt only.
6. To disseminate result.

CHAPTER TWO

LITERATURE REVIEW

2.1 MICRONUTRIENTS DEFICIENCIES

About 1.6 billion people in more than one hundred countries including Ghana suffer the consequences of micro nutrient deficiencies, notably iron, iodine and vitamin A. The consequences include blindness induced by vitamin A deficiency, mental and physical retardation caused by iodine deficiency, and impaired motor development and maternal mortality from iron deficiency.

2.1 IODINE

Iodine is an essential nutrient for human beings; its sole function in the human organism is its role in the formation of thyroid hormone, of which it is a basic component. Thyroid hormone regulates the metabolic rate of the individual. One of the factors, which affect the output of thyroid hormone by the thyroid gland, is availability of iodine. In the absence of sufficient iodine, the gland increases its secretory activity in an attempt to compensate for the deficiency; as a result, the gland enlarges and becomes turgid with an iodine poor

secretion. This condition is known as simple or endemic, goiter.

Synthesis of thyroid hormone may be effectively prevented, even in the presence of sufficient iodine in the diet and circulation, by certain drugs (prophythiouracil, methymazole, carbimazole), and by thiourea, all of which block the oxidation of iodine and iodination of tyrosine in the thyroid gland. Cabbage, rutabaga, Brussels sprouts, turnips and other members of the Brassica family contain a goitrogenic thiouxazolioden derivative which acts similarly in interfering with thyroid hormone synthesis. Under ordinary circumstances insufficient quantities of this goitrogens are ingested to be of clinical significant. Some toxic substances, such as thiocyanates, nitrates, and perchlorates, act by blocking the uptake of iodine ions by the thyroid gland.

2.2 IODINE REQUIREMENTS

About three-fourth of the 10 to 20mg of iodine in the adult body is found in the thyroid gland (which alone can synthesize thyroid hormone), with the rest concentrating in exocrine glands (salivary, mammary), the gastric mucosa, and certain other organs. The iodine requirement for prevention of goiter in adults is 50 - 75µg/day, or approximately 10g/kg body weight. The need for iodine is increased during puberty and pregnancy. Presumably the demand for thyroid hormone increases during these periods, and temporary enlargement of

the over secreting thyroid gland may be observed not infrequently. The need for iodine is increased during pregnancy because the fetus must derive its iodine requirements and stores from the mother. During lactation, maternal iodide is lost through secretion in the milk and the minimal iodine requirement is almost doubled at the time of maximal lactation. The iodine requirement for young infants is 40 μ g and 200 μ g in lactating mothers, (Burton and Foster, 1988).

Food, which is grown on iodine poor soil, contains insufficient iodine to meet human needs. Endemic goiter is observed frequently in regions deficient in iodine. A high incidence of goiter has been found in the mountainous areas in Ghana, and the hinterlands, (Asibey-Berko, 1991).

Among natural foods, the best sources of iodine are seafoods and vegetables grown on iodine-rich soils. Neither sources, enjoys universal distribution, and various methods have been proposed to ensure an adequate iodine intake, especially for populations in iodine-poor regions. The use of iodized salt has thus far proved the most successful and most widely adopted method.

2.3 IODINE DEFICIENCY

A thousand million people were estimated to be at risk of iodine deficiency alone in 1990. Two hundred million have goiters, 20 million are mentally retarded-six million of these being cretins, (PAMM 1992 Country Training Program Ending Hidden Hunger, 1991, ACC/SCN, 1992). Iodine is a public health problem in twenty-seven African countries with 227 million at risk in Africa alone, (ACC/SCN, 1992, FAO/WHO/ICN, 1992).

Within Ghana the National IDD survey found at least 33% of the country's 110 districts affected by serious IDD (technically describe as mild, moderate, and severe IDD), (Asibey-Berko and Orraca-Tetteh, 1994). IDD is the leading cause of intellectual and neurological impairment in the world today. Iodine deficiency leads to brain damage in the fetus, causing irreversible neurological damage to the brain. Its effect on the reproductive function are the increase rates of abortion, stillbirth, congenital abnormalities, low birth weights and infant mortality rates, (Ending Hunger, 1991). When the iodine level of the blood is low, the cells of the thyroid gland enlarge due to synthesis of a thyroxin precursor intended to trap as many particles of iodine as possible. When the gland enlarges until it is visible, it is called goiter. In addition to causing sluggishness and weight gain, a thyroid deficiency may have serious effects on the development of the infant in the uterus. Severe

iodine deficiency during pregnancy causes the extreme and irreversible mental and physical retardation known as cretinism. Much of the retardation can be averted by correction of the deficiency during pregnancy, but if it goes uncorrected, the child's intelligent quotient (IQ) may be as low as 20 percent, (Hamilton *et al.* 1995). Iodine deficiency may either result from inadequate dietary intake or presence of goitrogenous substances in the food. The goitrogens present in the food may inhibit the iodine in the food from being absorbed in the gut, resulting in deficiency.

TABLE 1: IDD SEVERITY AND THE NEED FOR CORRECTION
CLINICAL FEATURES

Goiter	Hypo thyroidism	Cretinism	Goiter Prevalence %	Mean Iodine $\mu\text{g}/\text{dl}$	Need for Correction
Mild +	0	0	10-30	3.5-5.0	important
Moderate ++	+	0	20-50	2.0-3.5	urgent
Severe +++	+++	++	30-100	<2.0	clinical

KEY : 0 = Absent, + = present but mild, ++ = moderate, and +++ = severe.

Source: Dunn *et al.* 1990, a practical guide to the correction of iodine deficiency.

2.4 IRON DEFICIENCY ANEMIA

Iron deficiency in human beings is expressed as hypochromic anemia, which

occurs most often in infants, young children, and women of childbearing age. In this condition the number of circulating red blood cells (r b c) is either normal or reduced, but the total quantity of circulating hemoglobin is decidedly subnormal. Each erythrocyte has reduced hemoglobin content, and the red blood cells are pale. The blood has a decreased oxygen-carrying capacity, which reflects unfavorably on most body functions. Iron deficiency anemia may develop on a purely nutritional basis, as a result of inadequate diet or poor absorption. Occult or overt blood loss accelerates the depletion of body iron. Even though absorption and the diet are adequate, chronic blood loss may lead to a severe exhaustion of the body's iron stores and to subsequent hypochromic anemia. Individuals who harbor intestinal parasites or who suffer from chronically bleeding hemorrhoids, peptic ulcers, or recurrent nosebleeds are particularly prone to iron depletion. Women in their reproductive years may develop a deficiency if their menstrual loss is copious or if repeated and closely spaced pregnancies recurrently exhaust their iron stores. A concurrent poor diet or iron absorption puts a strain on the already overburdened iron economy, and frank deficiency symptoms are not infrequent in this group.

In iron deficiency anemia (which is about half of several types of anemias), the red blood cells contain too little hemoglobin and become unable

to carry enough oxygen to meet the cell's energy requirements. Iron deficiency anemia (IDA) is the most widespread nutritional deficiency in the world today (Ending Hidden Hunger, 1991 /ACC/SCN, 1992/ FAO/WHO/ICN, 1992). Two billion people are affected by anemia, more than half being preventable and treatable anemia. Prevalence rates are higher in developing countries. The people most affected are women of childbearing age, and pre-school children. About 50% of women of childbearing age around the world are estimated anemic as a result of iron deficiency. Africa is leading with nearly 60% expectant mothers and children (0-5 years) affected (FAO/WHO/ICN, 1992). Iron deficiency if uncontrolled leads to increasingly severe anemia, reduced work capacity, reduced learning ability due to impaired cognitive development, increase susceptibility to infections and greater risk of complications or death of mothers during pregnancy and child birth. Roughly 20% of all maternal deaths in West Africa and India - when blood transfusion is not available- is directly attributable to anemia (Ending Hidden Hunger, 1991). In general this contribute to 20-40% of all maternal deaths in developing world.

In Ghana the inadequacy of dietary iron intake appear to exist around the country, especially among the rural and urban poor. Twenty three percent

of children admitted to Axim hospital in 1991 had severe anemia (Hb < 5g/dl). Ninety five percent of these were pre-school children (Addo, 1991, Nutrition Division Ministry of health, Ghana.) By WHO (1968) standards (Hb<11.0g/dl), 63.7% pregnant hospital attendants were found anemic in 1973 (n=1098). 69% of pregnant women were found anemic at antenatal clinics in 1987. In 1991, the Blood Transfusion services in Ghana found 40% of all its blood transfusion going to children because of high rates of anemia among them (Nutrition Division.) Regional data from the Ministry of health, Ghana in 1991 demonstrated proportionately high anemia prevalence among pregnant and lactating women. The data found 2.5 times more anemia prevalence among lactating women in comparison with pregnant women (Asibey-Berko, 1992). Malaria, intestinal worm infestation and other infections aggravate anemia in Ghana.

Table 2: Hemoglobin (Hb) Levels below which anemia is judged to be present.*

Age/ sex group	Hemoglobin level
Children 6 months- 5 years	< 11
Children 6 - 14 years	< 12
Adult males	< 13
Adult females (non-pregnant)	< 12
Adult females (pregnant)	< 11

*source: WHO/UNICEF/UNU (1997), adapted from WHO (1968).

Table 3: Degrees of anemia among children aged 6 months-5 years.*

Grades	Hemoglobin level (g/dl)
Mild	10
Moderate	7 – 10
Severe	7
Very severe (life threatening)	4.0

2.5 THE ROLE OF MALARIA IN IRON DEFICIENCY ANEMIA

Anemia is one of the most common manifestations of malaria. The causative mechanisms are multifactorial, with the severity of anemia varying in relation to the level of malaria transmission in a population (Brabin, 1992). Hemolysis leads to the loss and degradation of hemoglobin and hence anemia.

Malaria also appears to enhance iron deficiency. Apart from other factors, hemoglobinuria occurs during malaria, leading to a loss of hemoglobin and hence iron in the urine (Brabin, 1991).

The anemia of acute malaria in non-immune individual is usually normocytic and presents no special morphologic features; however, in malaria

endemic area subjects frequently have multiple pathology including iron and possibly folate deficiency. Interactions between nutritional factors and malaria infection in the causation of anemia are complex, (Weatherall, 1988). In addition, the stability of malaria in an area influences the risk of developing anemia. In unstable malaria areas anemia occurs in all ages, whereas in stable areas anemia is most evident in young children and pregnant women. Where transmission is seasonal, marked changes in prevalence in anemia may occur, especially in children, (McGregor *et. al*, 1966.)

2.6 CYCLE OF INTERACTION OF MALARIA AND NUTRITIONAL ANEMIA

In young children and pregnant women living in endemic areas, malaria-associated anemia may present with features compatible with iron deficiency and folate deficiency states (Draper, 1960). There is some evidence that persistent or recurrent parasitemia induces iron deficiency, although the mechanisms are uncertain. These include the following:

1. There is reduced absorption of iron during the acute period of the illness (Molyneux *et. al*, 1989).
2. Low haptoglobin levels, which result from intravascular hemolysis, will

reduce the formation of haptoglobin/hemoglobin complexes which are removed from the circulation by the liver, reducing iron availability (Boreham *et. al*, 1981). Once this happens the iron in any additional free hemoglobin will be lost to the body either acutely (as hemoglobinuria) or, more commonly, as hemosiderinuria over a longer period (Kariks, 1969).

3. There is immobilization of iron in hemozoin complexes (malaria pigment) (Abdalla, 1990 and Fulton and Maegraith, 1949).

The magnitude of such mechanisms in subjects experiencing chronic recurrent parasitemia is unknown. For example, in some individuals it may take up to 200 days before parasitemia is suppressed and under holoendemic conditions subjects may experience one or two infected mosquito bites each week. It is difficult to assess the contribution to malabsorption under such conditions of high transmission.

Comparison of the prevalence of iron deficiency in malarious and nonmalarious areas is affected by differences attributable to diet or other confounding factors.

Nevertheless, it is of interest that iron deficiency is a widespread cause of anemia in coastal Papua New Guinea, where malaria is endemic (Kariks 1969), but in the New Guinea highlands where it is not endemic, there is

evidence that iron deficiency is a much less frequent cause of anemia (Kariks *et al* 1972 and Bailey, 1966). Where iron status is already marginal, or latent iron deficiency is already present, the additional losses caused by malaria may be sufficient to tip the scales towards overt iron deficiency. In Liberia, significantly lower HbA levels were observed in children from malarious compared to the non-malarious areas; in addition, malaria prophylaxis led to an increase in the HbA levels, to values comparable with those from children in nonmalarious areas (Willcox *et al.*1985). The authors suggested that these findings may be attributable to iron deficiency caused by malaria.

Carefully conducted clinical studies in The Gambia by Abdalla 1990, have not implicated malaria directly as a cause of the high prevalence of iron deficiency observed. Iron deficiency could not be attributed as the sole cause of dyserythropiesis in patients with chronic malaria anemia. An earlier detailed study by Abdalla *et al.* 1980 also showed that the pathophysiological mechanisms responsible for the dyserythropoiesis of *P. falciparum* malaria are different at different stages of illness.

Hemolysis from malaria stimulates erythropoiesis and increases the requirements for folate (Strickland *et al.*1970). This conclusion is the bases

mainly on evidence from a clinical trial reported by Fleming *et al.* 1968, on antimalarial chemoprophylaxis in pregnant women in northern Nigeria. Serum folate activity fell more rapidly in pregnant women not protected from malaria by chemoprophylaxis. In this same population, protection from malaria has also been shown to prevent severe anemia and megaloblastosis in pregnancy even without the addition of a folate supplement (Fleming, 1968).

Megaloblastic anemia in infants and children other than the severely malnourished has been reported infrequently from tropical countries (Walt and Holman, 1956). This is surprising in view of the expected high prevalence of folate deficiency secondary to malarial hemolysis. Infants and young children experience high attack rates from malaria and show much higher parasite densities than do adults (Brabin, 1990), and there is no adequate explanation of why folate deficiency anemia is not more frequent if malaria is a significant contributory factor. Megaloblasts have been reported in the bone marrow in a selected group of anemic children in Zaria, northern Nigeria, in whom it was estimated that 40% had presumed folate deficiency. Malaria was diagnosed in 74% of those with megaloblastic erythropoiesis (Fleming and Werblinska, 1982). However, a proportion of these children had sickle cell disease and it was not clear

what percentage had dyserythropoiesis, as described by Abdalla and Weatherall (Abdalla *et al.* 1980) in anemic children with malaria.

Other studies in Nigeria indicate that iron deficiency is of greater importance in children than folate deficiency, which was not considered a major cause of anemia (Akenzua *et. al* 1985). In north Transvaal, South Africa, 25% of 3-to 5-year-old children had red cell folate activities below normal and none had high folate values, but malaria is not endemic in this area (Van der Westhuyzen *et al.* 1986).

Iron deficiency impairs host-cell-mediated immunity (Joynson *et. al* 1972 and Macdonald *et. al* 1975) and phagocytosis (Chandra, 1973). Research is required to investigate the associations between maternal malaria and iron status and neonatal and infant anemia.

2.7 ANEMIA AND PROTEIN-ENERGY MALNUTRITION

Hematological disturbances and various types of anemia have been found to be associated with victims of protein-energy malnutrition, (PEM). Commonly there is a hypochromic, microcytic anemia of the type seen in iron deficiency anemia, yet it is sometimes refractory to normal therapeutic regimens of

anemia. As a result of the obscurity of the exact nature of the anemia of PEM, many studies have been undertaken in an attempt to elucidate its mechanism. Lynch *et al.* 1967 have explored the possibility that hemolysis caused anemia in these children. In their study of marasmic children they found no decreased survival of red cells, but they stated that there have been some reports of hemolysis as a factor in typical kwashiorkor patients.

A study of the absorption of iron in kwashiorkor patients revealed that iron in the ferrous form was generally well absorbed (greater than 24 percent in four out of five patients), but iron in the ferric form was poorly absorbed (1-10 percent), (Lynch *et. al* 1967). This behavior is quite normal, since ferric iron generally has a much lower bioavailability than ferrous iron.

2.8 DELETERIOUS EFFECT OF ANAEMIA

a) Effect on Cognitive ability:

Iron deficiency, if not corrected leads to severe anemia and this has been shown conclusively in the field studies in many countries to delay psychomotor development and impair the cognitive performance of infants in Chile, Costa Rica, Guatemala and Indonesia, and the pre-school children of Egypt, India, Indonesia, Thailand and the United States (WHO Technical Report series No. 477, 1971/8th Report of the joint FAO/WHO Expert

Committee, 1991).

b) Resistance to Infection

Morbidity from infectious diseases is increased in iron deficient populations because the immune system is adversely affected. In the presence of anemia leukocytes (W. B. Cs) have a reduced capacity to kill infested micro-organism, lower concentration of cells responsible for immunity and depressed skin test response to common antigens. Iron supplementation of deficient children and the fortification of their milk or cereal have shown to reduce their infectious illness.

c) Work Capacity and Productivity

A positive linear relationship between iron deficiency and work capacity has been demonstrated from agricultural workers in Colombia, Guatemala, Indonesia, Kenya and Sri Lanka (Scrimshaw, 1991.) Work capacity is drastically reduced during iron deficiency but returns rapidly to normal with iron supplementation.

d) Pregnancy Outcome

Iron deficiency in child bearing women increases maternal mortality, perinatal

and prenatal, infant mortality and premature and low birth weight infants. Anemia in pregnancy also favors many complications like excessive bleeding before, during, and after labor. Anemic women are 5-10 times more likely to die in childbirth than non-anemic ones (Bailey, 1994). About 40% of all maternal perinatal deaths are linked to anemia. Favorable pregnancy outcomes are 30% to 45% less prevalent in anemic mothers, and their infants have less than one-half the normal iron reserves. Such infants require more iron than supplied by the breast milk at an early age than infants of normal birth weight do.

At childbirth anemia may raise the risk of maternal death by 500% and infant death by 600%.

e) **Heavy Metal Absorption**

An important consequence of iron deficiency is an increase risk of lead poisoning in a child exposed to high levels from chipped lead paints or other environmental exposure to excessive lead. Increase lead levels are found in children in many cities with high lead pollution from automobile fumes. Prevention of iron deficiency reduces the number of children susceptible to lead poisoning through greater lead absorption and may help reduce their lead

burden.

It has been pointed out that the elimination of iron deficiency alone can do more than any single program to achieve the goals of development because all sexes and age groups are affected (FAO/WHO/ICN, 1992).

2.9 INTERVENTIONS IN IRON DEFICIENCY

Given the state of the people and the present knowledge of iron metabolism and iron deficiency, the question remains: how can we get iron to those who lack it, and how are we improving the quality of their lives by doing so? Very few large field trials have been conducted with respect to iron nutrition; most studies have been academic projects using fairly small groups. Both types give some clues to what might be possible in the future.

2.9.1 DIRECT IRON SUPPLEMENTATION

It is clear, from many sources that it is entirely possible to raise hemoglobin levels using direct iron supplement. For a faster result, intramuscular injection is given. A strategy for prevention of iron deficiency is weekly iron supplementation. The argument in support of intermittent supplementation is derived from studies in rats showing that iron absorption is inhibited when preceded by massive doses of oral iron. The mucosal block to iron absorption is assumed to occur with daily doses. Theoretically less frequent administration of iron should enhance absorption sufficiently to offset the reduction in total iron dose. In rats administration of iron supplements every third day is as effective as daily supplementation (Viteri *et al.* 1995). There

are few published clinical trials comparing the effect on iron status between daily and less frequent supplementation schedules. The studies that have been completed are primarily in adults (non-anemic women, anemic women and pregnant women) and the outcomes have been contradictory. Higher absorption of iron, when given on once-a-week basis could not be confirmed in non-anemic American women (Cook and Reddy, 1995). On the other hand, studies in non-anemic pregnant and anemic pregnant Indonesian women demonstrated similar efficaciousness of weekly supplement as compared to daily supplementation in restoring or maintaining hemoglobin concentrations (Gross and Schultink *et al.* 1994, and Ridwan, Schultink *et al.* 1996). A review of the literature revealed two studies on intermittent iron supplementation in pre-school children (Schultink and Gross *et al.* 1995, and Liu and Kang, 1995).

The efficacy of intermittent iron supplementation was evaluated recently by Schultink *et al.* 1995, who compared daily with twice weekly iron administration in a randomized double-blinded field trial on 87 two to five year old children in Jakarta (Punnonen *et al.* 1994). Children were randomly assigned to receive 30mg iron as liquid ferrous sulphate either daily or twice weekly for two months. Baseline hemoglobin values were less than 112g/l. Correction of iron deficiency as measured by an increase in hemoglobin or

decrease in free erythrocyte protoporphyrin was two fold greater in 32 children given daily than in 33 children given iron twice a week. However after statistical adjustment of the lower initial hemoglobin concentration in the children given iron daily, the difference was not statistically significant.

2.9.2 IRON SUPPLEMENTATION IN FOODS

The addition of iron to foods in the hope of preventing anemia has been in practice in certain parts of the world for years. Nonetheless, no thorough evaluations of the effectiveness of this measure have yet been carried out. Proposals for fortification of food products are aimed at two types of food: first, foods eaten only by specific target groups in the population (e. g infant formulas) and second, foods consumed by widest possible segments of the population (e. g wheat flour products). None of these interventions is designed to cure anemia where it is endemic and of severe proportions, and they may therefore be of least utility precisely where the problem is most urgent.

Some small studies have tested the effectiveness of iron-fortified formulas in preventing anemias of infancy. In one of such studies, three groups of babies were fed iron-fortified humanized milk, unfortified humanized milk, and isocaloric sweetened condensed milk respectively.

Hemoglobins in the first group were on the average, higher than those in the third group. The results were statistically significant only between the first and the third groups. The study showed that not only were hemoglobin levels up, but infections were down 50 per cent in the supplemented babies Schultink *et al.*1995. This finding alone is strong incentive for looking into the matter more carefully.

Supplementation of entire populations by adding iron to common staples is well known but poorly studied. Lately, opponents of this type of fortification have raised two medical issues: one, that a certain number of the people will be adversely affected by additional iron and will develop hemochromatosis and hemosiderosis with serious deleterious results; and two, that anemia as an early sign of carcinoma of the bowel would be masked by the extra dietary iron. Neither of these contentions has ever been shown to be fact. It is not known if relatively modest increases in dietary iron would really increase the incidence of hemosiderosis—an exceedingly rare condition, at best—or if the condition is already precipitated in all those at risk by the average iron intake of an ordinary, unsupplemented diet. As for cancer of the bowel, it is not clear that increments in dietary iron in non-therapeutic amounts could really mask anemia or that cases of cancer would be missed, which would otherwise be diagnosed.

The nature of technology in this type of supplementation is one of the biggest problems. The soluble salts of iron have the highest bioavailability but are used relatively infrequent because they decrease the storage stability of unbaked wheat flour, and must be added only at the time of baking. The effect on unbaked wheat flour is accentuated by high temperature and humidity and would therefore present an even more serious problem in the tropics, areas with serious problems of parasitism and anemia. Reduced or elemental iron is fairly well absorbed but gives a gray color to baked products and is not looked upon with favor by manufacturers.

Food fortification, in general, appears to hold promise for programs in the prevention—not curative—of anemia. However, until the technological problems are better worked out and the success of such strategies is evaluated, the mounting of effective supplementation programs will be problematical

2.9.3 SERUM FERRITIN

The body of an average adult contains about 3.5 to 4.0 g of iron. Of this, approximately 1g is stored iron, located mainly in the liver, spleen, and the bone marrow (Burton and Foster 1988). Reservoir iron is found intracellularly in the form of a protein complex, either as hemosiderin or as ferritin.

Hemosiderin is a complex substance made up of ferritin molecules and serves as an insoluble storage form of iron in the cell. Ferritin itself consists of a core of ferric hydroxide-phosphate (about 5000 iron atoms at saturation) (Burton and Foster, 1988) surrounded by 24 identical protein units. This iron is readily mobilized when the need arises. As a deficiency develops, the ferritin and the hemosiderin reservoirs are depleted, then there is decreased transport of iron (determined by percentage saturation of transferrin), and finally an acute anemia develops, with impaired hemoglobin production. The small amounts of serum ferritin that circulate in plasma are in equilibrium with tissue iron stores. The serum ferritin concentration is therefore an especially useful indicator of total body iron status. The reference interval is 15 to 200 μ g/l in men and 12 to 150 μ g/l in women (Kaplan and Pesce, 1989).

A low ferritin concentration is diagnostic of iron deficiency. Ferritin levels drop in the early development of iron deficiency, before serum iron and transferrin saturation become abnormally low. An increase in serum ferritin is seen in iron overload, before the development of signs and symptoms of hemochromatosis. However, the release of ferritin from damaged tissue in hepatitis, acute inflammatory conditions, and a variety of tumors also drastically increase the ferritin levels. Increases in serum ferritin must therefore be interpreted in light of the person's overall health.

2.10 IRON AND IODINE FORTIFIED SALT

The manufacture and distribution of salt fortified with iron and iodine has been suggested as inexpensive, effective and efficacious means of preventing iron and iodine deficiency disorders. A process of preparing a stable form of salt fortified with iron and iodine has been achieved at the University of Toronto, Department of Engineering, by Diosady, 1995. In this study 1000 ppm of iron in the form of ferrous fumarate and 50 ppm of iodine in the form of potassium iodide was used. Dextrin was used to encapsulate the iodine and prevent it from interacting with moisture and iron. The stability and bioavailability of the salt investigated has proved to be quite satisfactory (Zlotkin *et. al*, 1995).

CHAPTER THREE

METHODOLOGY

The study was conducted in two phases; the Acceptability study in phase one (preparation towards the Efficacy study) and the Efficacy study in the second phase.

3.1 Phase one: Acceptability Study:

This phase involved

1. Preparation of questionnaires
2. Preparation of some common Ghanaian dishes to evaluate the Acceptability of the salts
3. Selection of Panelists to evaluate the effects of the salts on the dishes
4. Statistical analysis of the results

3.1.1 Preparation of questionnaire

Multiple paired- comparison tests were considered as the most suitable sensory evaluation test for this type of evaluation. A questionnaire used for this kind of study was accordingly prepared. (Appendix 1). Three properties of the salts in the food and the raw salt (taste, appearance and overall acceptability) were to be tested. Overall acceptability here means all elements of sensory

evaluation such as texture, flavor, taste and appearance. The questionnaire comprised two tables. Table 1 (Appendix 1) allowed panelists to only say if any of the four salts in a dish made it

- a) Unacceptable in comparison with the reference salt (R), (Score = 1). The reference salt being a sample of ordinary uniodated Ghanaian salt from the market.
- b) Just as acceptable as R, (no difference, score = 5).
- c) More acceptable than R (Score = 9).

After this preliminary scoring, it is now easier for the panelists to score the same food again on table 2 (Appendix 2), zeroing-in on exactly how each dish with the salts differ from the dish containing the reference or local salt. Since table 2 gave more detailed evaluation of the salts, the data generated from it represented the findings from the study.

3.1.2 Selection and Preparation of some common Ghanaian dishes to which the study salts were added

Ten dishes popular across Ghana were selected. Selection was based on Ghanaian field data compiled by Asibey-Berko *et. al* 1997, showing the most frequent consumed dishes in different regions of Ghana.

The ten dishes include; rice and stew, koko, banku, light soup, yam ampesi

and kontomire stew, Ga kenkey, Tuo zaafi and ayoyo stew, okro stew, boiled apentu and boiled apem. The last two are different varieties of plantain.

The acceptability of the salts in their raw state was also evaluated at the beginning and at the end of the study.

Four different types of salts labeled A, B, C, & D among which the double fortified salt was included, together with the local salt as the reference salt (R) were used in seasoning the dishes. Ten people were selected to cook each of the ten dishes. Portions of each dish were seasoned with the four salts A, B, C, & D, including R. The same Ghanaian cooking methods were used for all the meals. Each salt sample was added to portions of the meal to taste, but aimed at adding approximately the same quantities of each type of the salt, to the portions. Different set of people were also selected to cook the same types of dishes for a replicate results.

3.1.3 Selection of panelists and sensory testing

Nine trained panelists were selected to evaluate the salts in the different dishes. For each meal, the panelists were each served five plates, four of which were labeled with a code number representing salts A, B, C, or D. The local salt was also coded R.

Panelists were asked to compare the content of each plate to that of the control (R) in terms of taste, appearance and overall acceptability. Scores were entered onto score sheets (Tables 1 & 2, Appendix 1)

Scoring on Table 2 was as follows:

Extremely unacceptable compared to R = 1

Very unacceptability compared to R = 2

Moderately unacceptable compared to R = 3

Slightly unacceptable compared to R = 4

No difference (that is, as acceptable as control) = 5

Slightly more acceptable (preferred to R) = 6

Moderately acceptable (preferred to R) = 7

Very acceptable (much more acceptable than R) = 8

Extremely acceptable (very much acceptable than R) = 9

The study was repeated using different set of cooks but the same panelists. The five raw salts were also evaluated at the beginning and the end of the study. Opened sachets of the salts at the start of the study were kept in Tupperware containers till the end of the study for the evaluation of the raw salt. The covers of the containers were left ajar to simulate what may be happening in the rural Ghanaian homes.

3.1.4 Data Analysis

Data from the detailed scoring sheets (Table 2, Appendix 1) was used for the statistical analysis. Analysis of variance ANOVA and multiple comparison test were used to analyzed the data, using Statgraphics software

3.2 PHASE TWO: THE EFFICACY STUDY.

This phase involved:

1. Administration of questionnaires to select the study children (initial screening) whose parents satisfied all inclusion criteria.
2. Initial hematological screening (baseline screening) from above children to select those with Hb \geq 10g/dl.
3. Assigning 150 selected children to the salt treatment groups (red, green and yellow -labelled salts) for four months.
4. Monitoring their health, compliance of salt usage, constant salt supplies the acceptability and any side effect of the salts.
5. A three-day salt consumption study.
6. Final or fourth month hematological study.
7. Laboratory analyses.
8. Statistical analyses.

3.2.1 Selection of study children

The study was conducted in the Sekyere West district of Ghana where the National Iodine Deficiency Disorders (IDD) survey found high IDD endemic (29.3% goiter prevalence and 2.7 μ g/dl mean population urine iodine excretion, Asibey-Berko, E. and Oracca-Tetteh 1994).

A questionnaire was used to select the children (Community screening form, Appendix 2).

The following Inclusion and Exclusion criteria were used in selecting the children.

a) Inclusion Criteria

1. Child was between 1-5 years old in the selected communities
2. Child's mother was also involved in the study.
3. The mother was responsible for preparing the family's food.
4. The mother provided an informed consent on the child's behalf and on her own behalf to participate in the study.
5. The child was to stay in the community throughout the study period.
6. The child's hemoglobin was not less than 10 g/dL

b) Exclusion Criteria

1. A child who was not available for the baseline hematological screening.
2. Child's hemoglobin less than 10 g/dL.

c) Sample size calculation

$$\text{Sample size Estimate (n)} = \frac{(2^2) (1.96^2) (SD^2)}{(\text{Desired change in outcome}^2)}$$

SD = standard deviation of the outcome in the study population.

Based on the paper by Punnonen, (Punnonen, 1994) the variance of hemoglobin (Hb) of normal adults is 13 (Hb SD = 13) and those with iron deficiency anemia (Hb SD = 19).

The expected range in variance of hemoglobin is 13^2 - 19^2 .

Using the above equation and assuming for hemoglobin change of greater than or equal to 1.0 g/dL over the four-month intervention, the sample size range was 26 - 55 subjects per group.

Based on data supplied by Asibey-Berko from the Sekyere West District of Ghana where the National Iodine Deficiency Disorders (IDD) survey found a high IDD rate (Asibey-Berko E. and Orarca-Tetteh, 1994), the urinary excretion of iodine was $2.7 \pm 4.7 \mu\text{g/dl}$.

Normal urine iodine values are 10 $\mu\text{g}/\text{dl}$. Thus assuming a variance of 4.73^2 and a desired change of 7.3 $\mu\text{g}/\text{dl}$, the sample size was estimated to be 7 subjects per group. Based on the inclusion and exclusion criteria and the sample size calculation a sample size of 50 children per group were selected.

The communities involved in the study were Abuonem, Adidwan, Apaa, Atonsuoagya, Hweddiem, Kyekyewere, Nintin, Nkwanta and Yonso.

3.2.2 Initial Hematological screening

A total of 289 children aged between 1-5 years were initially screened at baseline for hemoglobin value greater than or equal to 10 g/dl. Out of this a total number of 159 children had their hemoglobin greater than or equal to 10 g/dL. Those with hemoglobin less than 10 g/dl were eliminated from the study but were given vitamin B complex syrup, FAC (Ferrous Ammonium Citrate) syrup and fersolate syrup to treat their anemia and those who required blood transfusion were advised to do so. One hundred and fifty (150) children were selected for the study.

3.2.3 Assigning of the selected subjects to the treatment groups

The salts were labeled Red, Green & Yellow, one of which contained both the iron/iodine (fortified salt) and the two other were iodized salt.

DOUBLE-FORTIFIED SALT CONTAINED	IODISED SALT) CONTAINED
1000 ppm iron as ferrous fumarate and 50 ppm iodine as potassium iodide	50 ppm Potassium iodate

Those selected were assigned to one of the three intervention groups in a randomized controlled double-blind design for four months. The treatment groups were 1) Red-labelled salt (R), 2) Yellow-labelled salt (Y) and Green-labelled salt (G). The double fortified salt contained 1000 ppm iron as ferrous fumarate and 50 ppm iodine as potassium iodide (Table above). An average daily intake of the salt of 10g/day was estimated to provide 70mg iron per week. The iodized salt provided equivalent iodine as potassium iodate.

3.2.4 Monitoring the health of the subjects and Compliance of the salt usage

The health of the subjects, side effects and the compliance with usage of the salts were monitored weekly, using the Weekly Health Monitoring questionnaire (Appendix 3). It was also ensured that the subjects received constant supply of salt to avoid using any other salt apart from the study salt.

This was done by weekly visits to the homes of the subjects.

3.2.5 Salt Consumption Study

A three day salt consumption study was done to assess the actual quantities of salt and hence iron and iodine being ingested daily. The study was conducted in two of the nine communities in the study area, namely, Abuontem and Nintin. The three-day salt intake assessment was done at the end of the second month of the study. Methodology used to measure the per capita salt consumption during the National IDD survey (Asibey-Berko, and Oracca-Tetteh, 1994), was employed. (Appendix 4).

3.2.6 Data collection

Following screening for inclusion as above, blood was collected from the children for the determination of hemoglobin, ferritin malaria parasite burden and sickle cell. Urine was also collected for urinary iodine determination at baseline, and the fourth month of study.

3.2.7 Laboratory procedures

a) Blood sampling

About 5ml venous blood was collected from each subject into a blood collection tube. Alcohol (70% Isopropanol) with a cotton ball was used to clean the arm of each child before blood was taken. This was done to prevent any infection. The blood sample collected was used for the following determinations.

i) Malaria parasite determination

A blood smear was prepared, using a drop of blood on a microscope slide. After drying it was immersed and stained with germicide stain for detection of parasites. Another drop was also put on a fresh microscope slide for sickle cell determination. The sample collected into the blood collection tube was sent to the laboratory centrifuged at 3000rpm for ten minutes and the serum collected into a tube and frozen at -20°C in a freezer for ferritin determination.

ii) Sickle cell determination

Reagent used: 20g of Sodium dithionite (Sodium hydrosulphite) was weighed and dissolved in 1000ml of distilled water.

Procedure:

To one drop of fresh blood sample on a microscope slide, two drops of the solution was added and covered gently with a cover slip. After about ten minutes, the prepared slide was read under the microscope for sickle cells or normal cells

iii) Hemoglobin Determination

Hemoglobin determination was done on the field using a drop of the venous blood collected. The blood was drawn into a Hemocue cuvette by capillary action and read directly in a Hemocue within ten seconds. (AM. Clinical Products Review, April, 1987). The system consisted of disposable micro-cuvettes, which contained reagents in dried form and a portable direct-reading haemoglobinometer. It was read in the haemoglobinometer at 565 and 880nm, the absorbance were made within 10 minutes to avoid error. The dried reagents in the microcuvettes comprised of sodium desoxycholate which hemolyses the erythrocytes, sodium Nitrite which oxidizes hemoglobin and sodium azide to convert Methemoglobin (Hi) to Methemoglobin azide (HiN₃) and sodium fluorescein which lines the inner surface of microcuvettes to standardize the light path length in the cuvettes.

iv) **Serum Ferritin**

Measurement was made with an Elisa technique, (Fidenza, 1991). Microtitre plates were coated with antiserum to human ferritin and incubated overnight at 4°C. The plates were washed, dried and the ferritin standards or unknown sera added and incubated at 37°C for two hours, causing the ferritin and the antibody coating to bind. After rinsing the wells and drying, anti-ferritin immunoglobulin previously conjugated with horseradish peroxidase (HRP) was added and incubated for one hour at 37°C. The ferritin being assayed was sandwiched between the solid phase and the indicator antibody at this stage. The wells were washed again and dried. The amount of enzyme retained in each well was measured by adding O-Phenylenediamine (OPD) substrate and incubated for 10 minutes at room temperature. The reaction was stopped by sulphuric acid and the absorbance in each well (proportional to ferritin concentration) read at 450nm. Concentrations were read from a standard curve.

b) **Urine Iodine determination**

Urine collection tubes were given to the subjects to provide urine for urinary

iodine determination. An amount of 0.1 ml of concentrated acetic acid was put in each of the urine tubes to preserve the urine on the field. The samples were later frozen in a freezer for laboratory analysis.

Urine iodine determination was done on a random urine specimen collected from each of the children.

i) Procedure for urine iodine determination:

An acid digestion method by Dunn et al. 1993 was used for the urine iodine determination. The iodine content was measured by its catalytic action in the reduction of Ceric Ammonium Sulphate (yellow) to the Cerous form (colorless). Absorption was measured in a Shimadzu 120 spectrophotometer. Samples were vortexed to mix and 250 μ L of each sample pipetted into test tubes. Serial dilutions of 0, 10, 20, 40, and 60 μ L of the standard 'B' solution were prepared in duplicates in 10 other test tubes containing 250, 240, 210 and 190 μ L of water respectively, to give a volume of 250 μ L in each test tube. This gave a standard curve of ranging from 0 to 120 μ g/L. Seven hundred and fifty (750) μ L of chloric acid was later added to each tube and vortexed to mix gently. The tubes were then heated (digested) for 50 minutes at 110-150 $^{\circ}$ c in a fume chamber. The tubes were cooled to room temperature after heating.

Three and half (3.5) ml Asernic acid solution was added to each tube, mixed by vortex and allow to stand for 15 minutes. Ceric ammonium solution (400 μ L) was then added to each tube at an interval of 30 seconds, mixing each by vortex. Thirty minutes after the addition of the ceric ammonium sulphate to the first tube, the absorbance was read at 405nm, and the rest of tubes were also read in the order by which the ceric ammonium solution was added , at an interval of 30 seconds.

ii) Calculation of Results for urine iodine

A standard curve of the absorbance at 405nm versus the standard iodine concentration was plotted. The corresponding iodine concentrations of the samples were determined. This was made possible by a multicalc software, a laboratory computer analytical package.

3.2.8 Statistical analysis

The absolute values and change in hemoglobin and other hematological indicators and urinary iodine at baseline and the fourth month were statistically analyzed using ANOVA by using EpiInfo and Excell soft wares.

CHAPTER FOUR

RESULTS

4.1 THE ACCEPTABILITY

The table below shows the acceptability of four salt types labelled A, B, C, and D assessed by nine judges in terms of taste, appearance and overall acceptability in some common Ghanaian dishes.

TABLE 4: ACCEPTABILITY OF DOUBLE FORTIFIED (A) SALT COMPARED TO FOUR OTHER SALT TYPES INCLUDING A LOCAL SALT 'R' IN SOME COMMON GHANAIAN DISHES

DISH	SALT TYPE	TASTE M±SD	APPEARANCE M±SD	OVERALL ACCEPTABILITY M±SD
1. PLAN-TAIN (BOILED)	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	6.3±0.3	1.±0.3**	4.0±0.1
	B	6.4±0.2	5.6±0.4	6.0±0.3
	C	5.7±0.5	5.3±0.5	5.3±0.2
	D	6.9±0.1	5.8±0.3	6.1±0.2
2. YAM AMPESI &KONTO- MIRE STEW	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	4.7±0.5	5.3±0.4	4.2±0.4
	B	5.4±0.4	5.6±0.3	5.6±0.4
	C	5.3±0.4	5.2±0.4	5.2±0.4
	D	6.3±0.4	5.2±0.3	6.2±0.4
3. OKRO STEW	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.1±0.4	3.0±0.2**	4.1±0.2*
	B	5.5±0.4	5.4±0.2	5.2±0.4
	C	5.7±0.3	5.3±0.2	5.5±0.7
	D	5.6±0.3	5.1±0.2	5.4±0.1
4. BANKU	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.1±0.1	5.1±0.2	5.4±0.3
	B	5.2±0.3	4.8±0.3	5.2±0.5
	C	5.0±0.0	5.1±0.1	5.3±0.3
	D	5.1±0.1	5.2±0.2	5.3±0.2

* significant difference ($p < 0.05$)

** Highly significant ($p < 0.01$)

M±SD = Mean ± Standard Deviation

Cont. of table 4

DISH	SALT TYPE	TASTE M±SD	APPEARANCE M±SD	OVERALL ACCEPTA-BILITY M±SD
5. RICE AND STEW	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.4±0.4	5.4±0.3	5.7±0.3
	B	5.4±0.3	5.5±0.3	5.6±0.3
	C	5.4±0.4	5.2±0.3	5.2±0.3
	D	5.3±0.3	5.4±0.3	5.6±0.2
6. LIGHT SOUP	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.0±0.2	5.1±0.1	5.4±0.2
	B	5.4±0.4	5.0±0.1	5.5±0.3
	C	5.3±0.3	5.2±0.2	5.5±0.2
	D	5.1±0.4	5.1±0.2	5.1±0.2
7. TUO ZAAFI & AYOYO SOUP	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.2±0.4	5.2±0.1	5.5±0.2
	B	5.8±0.2	5.6±0.3	6.1±0.3
	C	5.7±0.4	5.7±0.1	5.7±0.3
	D	5.8±0.4	5.3±0.5	5.7±0.3

* significant difference ($p < 0.05$)** Highly significant ($p < 0.01$)

M±SD = Mean ± Standard Deviation

Cont of Table 4

DISH	SALT TYPE	TASTE M±SD	APPEARANCE M±SD	OVERALL ACCEPTA-BILITY M±SD
8. BOILED APEM	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.8±0.4	1.9±0.3**	5.0±0.5
	B	5.8±0.4	5.9±0.4	6.1±0.3
	C	5.6±0.4	5.7±0.3	6.0±0.3
	D	6.2±0.3	5.7±0.3	6.0±0.3
9. GA KENKEY	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.3±0.3	5.2±0.3	5.0±0.3
	B	5.8±0.3	5.8±0.4	6.0±0.4
	C	5.3±0.2	5.4±0.3	5.6±0.3
	D	5.1±0.2	4.9±0.2	5.3±0.2
10. KOKO	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.7±0.4	5.3±0.3	5.4±0.4
	B	5.8±0.5	5.7±0.4	5.7±0.4
	C	6.0±0.4	5.6±0.4	5.8±0.4
	D	5.3±0.4	5.3±0.3	5.2±0.5

M±SD = Mean ± Standard Deviation

**Significantly different (p=0.0) from all the other salt types including the local salt 'R'.

TABLE 4b: EVALUATION OF RAW SALTS

STUDY PERIOD	SALT TYPE	TASTE*	APPEARANCE*	OVERALL ACCEPTABILITY*
1. RAW SALT (Beginning of study)	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.3±0.2	4.7±0.5	4.8±0.3
	B	6.7±0.3	8.2±0.4	7.9±0.2
	C	6.8±0.4	7.9±0.3	7.8±0.3
	D	6.8±0.5	8.2±0.4	8.0±0.4
2. RAW SALT (End of study)	R	5.0±0.0	5.0±0.0	5.0±0.0
	A	5.4±0.7	3.0±0.6	3.4±0.4a
	B	6.1±0.2	7.1±0.6	6.8±0.5b
	C	6.5±0.4	7.0±0.4	6.9±0.4c
	D	6.8±0.4	7.0±0.4	6.0±0.5

* Mean ± Standard Deviation

**Highly Significantly different ($p=0.0$) from all the other salt types including the local salt 'R'.

a = Less Acceptable than R

b = Equally Acceptable as R

c = More Acceptable than R



From the statistical analysis, there was no statistical difference between all the salt types in terms of taste, appearance and overall acceptability in the dishes except boiled plantain and the okro stew. In boiled plantain, there was high significant difference in terms of the appearance ($p=0.0$) and the overall acceptability ($p=0.03$) between salt A and all the other salt types.

There was also significant difference between salt A and all the other salt, in terms of appearance ($p=0.0$) and overall acceptability ($p=0.04$).

In terms of taste, there was a 100% acceptance. In terms of appearance, the acceptability of salt 'A' yielded 90% acceptance.

THE EFFICACY STUDY

A total of 289 children were screened from nine communities in the Sekyere west district at the baseline for hemoglobin. The communities selected include Atonsuagya, Adidwan, Abuontem, Apana, Nkwanta, Kyekyewere, Hwedem, Nintin and Yonso. Out of the total number selected, 71% of them were found to be anemic $Hb < 11 \text{ g/dL}$. Thirty one (31) percent were found to have mild anemia ($Hb < 11 \text{ g/dL}$), 66.3% were moderately anemic ($Hb 7-9.9 \text{ g/dL}$), and 2.6% had severe anemia ($Hb < 7 \text{ g/dL}$).

The table 5 below shows the rate of anemia among the children in the various communities. The screening was done before the children were randomly assigned to the three salt groups.

TABLE 5: PREVALENCE OF ANEMIA IN THE SEKYERE WEST DISTRICTS AMONG CHILDREN OF 1-5 YEARS OLD AT INITIAL SCREENING

Community	Children at initial screening	No. of children with Hb < 11g/dL	% Rate of anemia among study children
Abuontem	36	31	86
Adidwan	39	28	72
Apaa	44	26	59
Atonsuoagya	46	37	80
Hwediem	08	5	63
Kyekyewere	30	20	67
Nintin	33	18	55
Nkwanta	20	13	65
Yonso	33	27	82
Total	289*	204	71

The table above shows the prevalence of anemia among the study children at the baseline. Abountem recorded the highest prevalence of anemia (86 %) among the children (1-5 years old), with Nintin having the lowest rate of anemia (55 %).

*289 = the total number of children screened at the baseline. Out of this

number, 150 children with Hb = 10g/dL were selected and assigned to the various salt types for the four- month study period.

The overall rate of incidence of anemia among children of 1-5 years old at the Sekyere west district was found to be 71% (Hb <11g/dL.

TABLE 6: DEGREES OF ANEMIA AMONG THE CHILDREN IN THE SEKYERE WEST DISTRICT

GRADES	HEMOGLOBIN (g/dL)	PERCENTAGE (%)
MILD	=10 < 11	46.0
MODERATE	7 – 10	51.45
SEVERE	< 7	2.55

The range of Hb levels for those who were severely anemic was 2.8-7.0g/dL.

Fig 2: Rate of anaemia among children of 1-5 years old in the Sekyere west district before salt treatment.

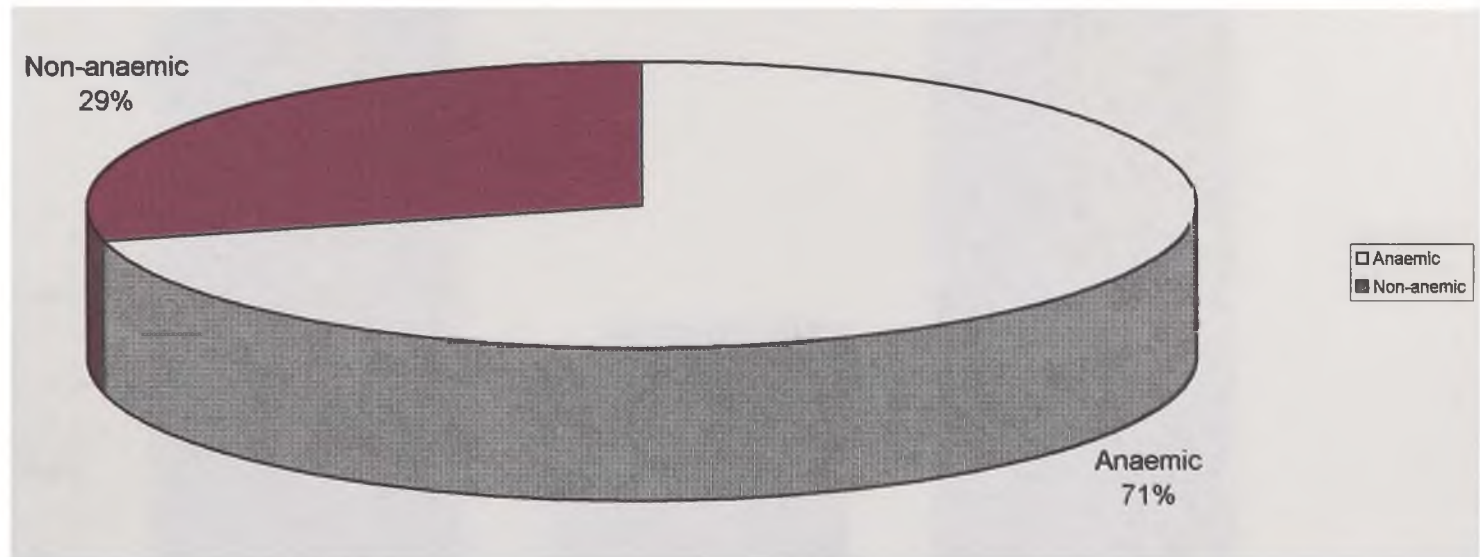
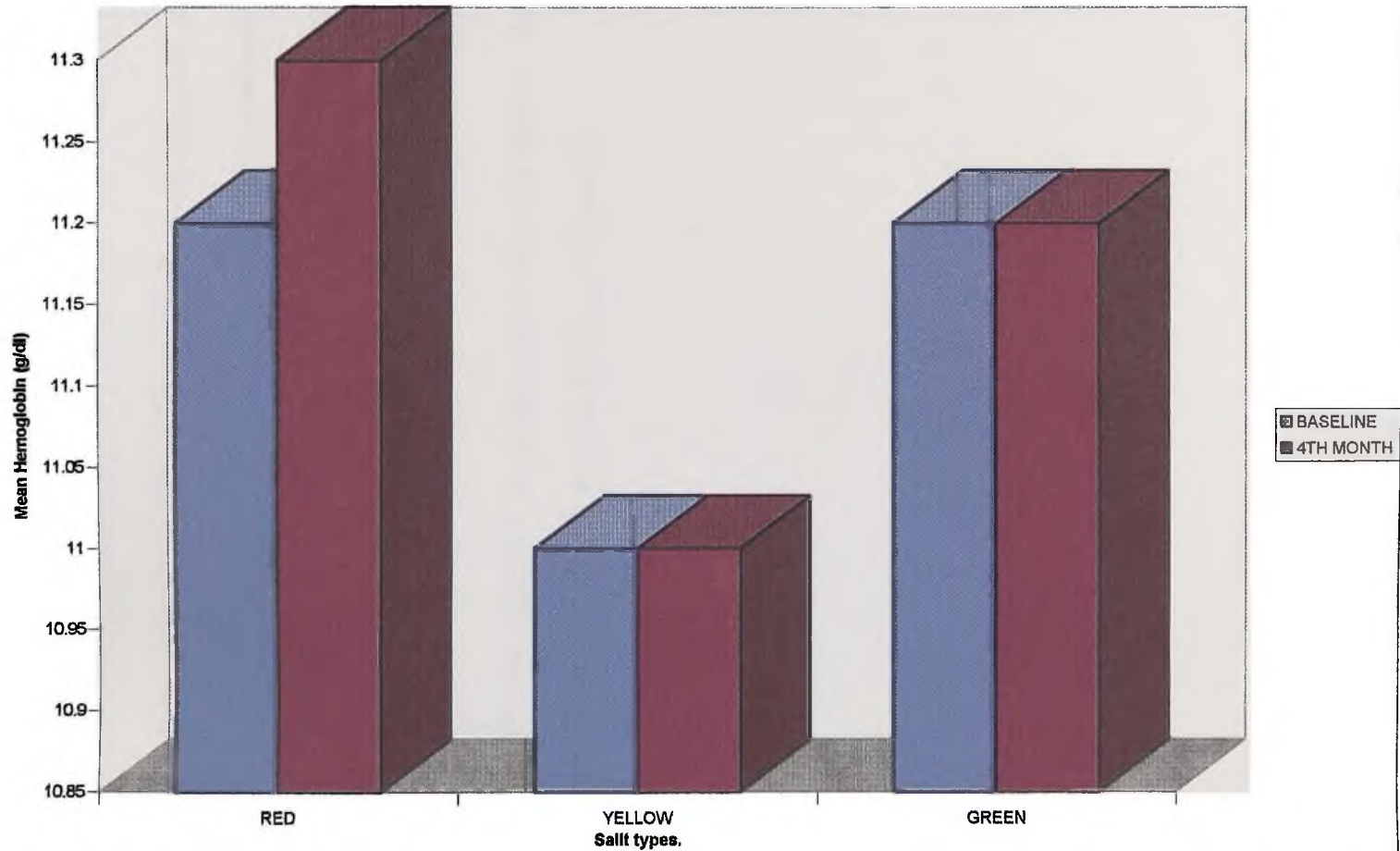


Fig 3: Comparison of mean Hemoglobin (g/dl) levels with salt types at baseline and 4th month of study.



Baseline mean hemoglobin = hemoglobin level before salt treatment
4th month mean hemoglobin = hemoglobin level after salt treatment.

Fig 2 above shows the mean hemoglobin values of the children both at the baseline and the fourth month of study. This hemoglobin represents the mean values of only children who qualified in the inclusion criteria ($Hb \geq 10$ g/dl). The fourth month hemoglobin was determined after the children were assigned to the different salt types for months. A comparison of mean Hb values for the three salts showed no significant difference ($p=0.8$). Again there was no significant difference at the fourth month ($p=0.4$).

FIG. 4
IODINE DEFICIENCY AT BASELINE ON THE BASIS OF URINE IODINE < 10ugd/L

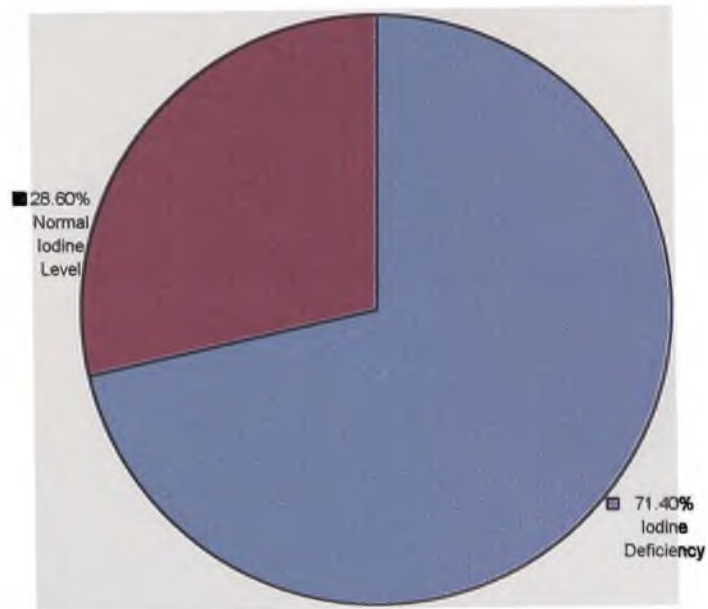


FIG. 5
DEGREES OF IODINE DEFICIENCY AT BASELINE

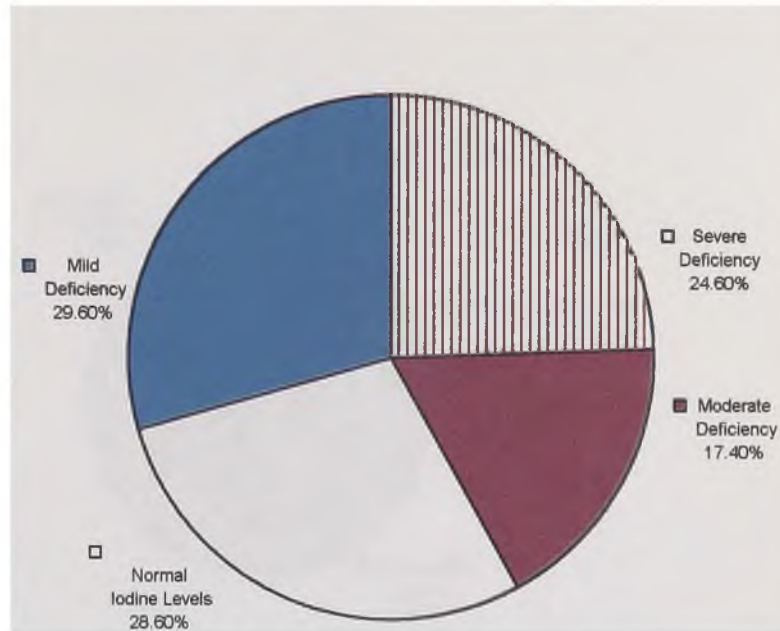


FIG. 6
Iodine Deficiency at 4th Month (Urine Iodine Concentration < 10ug/dL)

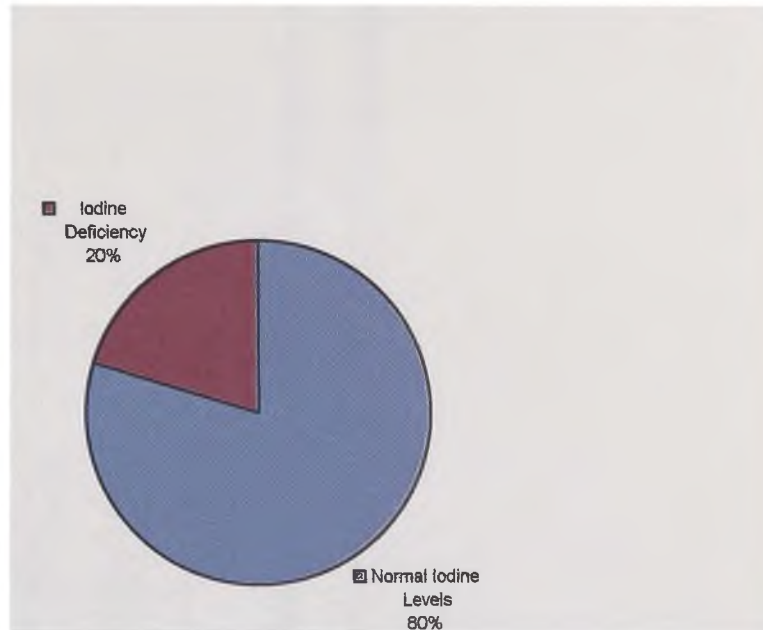


TABLE 7: COMPARISON BETWEEN THE HEMOGLOBINS OF BOY AND GIRLS AGED 1-5 YEARS

SEX	MEAN Hb	MEAN Hb
	(g/dL) AT BASELINE	(g/dL) AT 4 th MONTH
GIRLS	11.0± 0.9	11.1±1.8
BOYS	11.1± 0.8	11.3±1.8
TOTAL	11.0 ±0.4	11.2±1.4

There was no significant difference between the hemoglobin values of the boys and girls both at baseline ($p = 0.3$), and the 4th month of study ($p = 0.7$).

TABLE 8: PERCENTAGE OF CHILDREN WITH ANEMIA, IRON AND IODINE DEFICIENCY AT BASELINE

SALT TYPE	% With Anemia Hb<11g/ dL (n)	Mean Hb g/dL (n)	°Median serum ferritin µg/L (n)	% Iodine deficient subjects (UI) < 10µg/dL (n)	°Median UI µg/dL (n)
Red (R)	46 (37)	11.2 (37)	41.8 (30)	82.0 (37)	6.1 (37)
Yellow (Y)	56 (36)	11.0 (36)	37.4 (32)	72.7 (36)	3.5 (36)
Green (G)	36 (36)	11.2 (36)	54.8 (27)	66.0 (36)	6.7 (36)
Total	46	11.1	44.6	79.7	5.4

*Children with Hb = 10g/dL but < 11g/dL.

°Median was used instead of the mean because serum ferritin and urine iodine concentrations are not normally distributed.

Mean Hb values not significantly different by ANOVA ($p = 0.7$)

Ferritin values not significantly different by Kruskal-Wallis ($p = 0.1$)

Urine iodine values also not significantly different by Kruskal-Wallis

($p = 0.6$)

TABLE 9: PERCENTAGE OF CHILDREN WITH MILD ANEMIA, IRON AND IODINE DEFICIENCY AFTER FOUR MONTHS OF STUDY

SALT TYPE	% With Anemia* (Hb<11g/dL) (n)	Mean Hb g/dL (n)	°Median serum ferritin µg/L (n)	% Iodine deficient subjects (UI) < 10µg/dL (n)	°Median UI µg/dL (n)
Red (R)	19.0 (37)	11.3 (37)	67.6 (31)	15.0 (37)	27.6 (37)
Yellow (Y)	36.0 (36)	11.0 (36)	55.2 (32)	33.3 (36)	21.6 (36)
Green (G)	28.0 (36)	11.2 (36)	77.1 (26)	25.0 (36)	23.0 (36)
Total	27.5	11.2	66.6	20.3	24.6

° median was used instead of the mean because serum ferritin and urine iodine concentrations are not normally distributed.

*children with Hb = 10g/dL but < 11g/dL.

Mean Hb values not significantly different by ANOVA ($p = 0.5$)

Ferritin values not significantly different by Kruskal-Wallis ($p = 0.2$)

Urine iodine values also not significantly different by Kruskal-Wallis

($p = 0.9$).

TABLE 10: CHANGES IN BLOOD Hb, SERUM FERRITIN AND URINE IODINE (UI), LEVELS AFTER FOUR MONTHS OF SALT USAGE

SALT TYPE	*%with improved anemia status	Mean Hb Changed g/dL	Median ferritin $\mu\text{g/L}$	*%with improved Urine iodine status	Median Urine iodine $\mu\text{g/dL}$
RED (R)	27	+0.1	+25.8	67.0	+21.6
YELLOW (Y)	20	0.0	+17.8	39.4	+18.1
GREEN (G)	8	0.0	+22.3	41.0	+16.3

*percentage improvement found in hemoglobin (Hb) and urine iodine levels.

TABLE 11: AVERAGE SALT CONSUMPTION/ADULT/DAY IN A 3-DAY SALT CONSUMPTION STUDY

COMMUNITY	AVERAGE SALT PER ADULT /DAY (mean \pm SD)	RANGE
NINTIN	10.55 \pm 4.83	3.42-22.93
ABUONTEM	11.19 \pm 5.74	4.85-27.33
TOTAL	11.29 \pm 6.15	-

The average salt consumption of the people at the study area was found to be

11.29 \pm 6.15 g/day.

CHAPTER FIVE

DISCUSSIONS

5.1 EFFECT OF DOUBLE FORTIFIED SALT ON SOME COMMON GHANAIAIAN DISHES

Whenever iron and iodine are used to fortify any food substance, the iron in the ferrous state is oxidised to the ferric state. This makes it less available to the body and has an unpleasant taste and unattractive dark color. The iodine (iodide or iodate) is converted to elemental iodine, which sublimes and disappears rapidly, (Bagepallis, 1994). Scientists have tried over the years to fortify salt with both iron and iodine in a way that would prevent them from reacting and losing their potency.

In this study, four different salt types, labelled A, B, C and D, one of which was fortified with both iron and iodine were compared with a local (reference) salt also labelled R. The salts were used in cooking some common Ghanaian dishes. The purpose of the study was to find out whether or not the double fortified salt would impart any unpleasant color (appearance of the food) to any of the dishes. And also determine its acceptability (taste, appearance and overall acceptability). It was expected

that the ferrous in the double fortified salt would not be oxidised to the ferric state due to the new technology adopted in the fortification process.

a) EFFECT OF THE SALT ON TASTE OF THE DISHES:

The taste of all the dishes containing the four salts was found acceptable (100%). Compared to the reference salt (R), salts B, C, and D were more acceptable. However, salt A was acceptable as salt R.

b) EFFECT OF THE SALT ON APPEARANCE OF THE DISHES:

Salts B, C and D were found acceptable in all the dishes (100% acceptability). Salt A was only acceptable in seven (70%) out of the ten dishes. Salt A was found to darken boiled plantain, apem ampesi (apem is a variety of plantain) and okro soup. The darkening of the dishes may probably be due to the presence of polyphenol (polyphenoloxidase activity), (Owen and Fennema, 1985) in them. In the ferric state, an unattractive dark color develops. Plantain is suspected to contain a higher concentration of polyphenol than both okro soup and apem ampesi. The reason is that, the degree of the dark color developed in the plantain was more pronounced than the rest. It is suspected that polyphenol in the plantain, okro stew and apem ampesi reacted with the iron in the salt, thereby causing the darkening

of the food. Salt 'A' which imparted a dark color to these dishes was suspected to be the salt containing both the iron and iodine (double-fortified). Since plantain, apem ampesi and okro soup are suspected to contain polyphenols as a result of oxidation of iron which occurred and the dark color developed in these foods, it is advisable to eat such foods with iron absorption enhancing agents like vitamin C, heme protein and liver.

The result from the analysis of the raw salts (Table 4b) indicates that all the salts (in their raw state) were acceptable at the beginning and at the end of the study. However, salts 'B, C, and D' were found to be whiter and more acceptable than both salts 'A' and 'R' which were not significantly different ($p = 0.52$) from each other.

c) EFFECT OF THE DOUBLE FORTIFIED SALT ON THE OVERALL ACCEPTABILITY OF THE DISHES

The overall acceptability includes the aroma, texture, taste, and appearance of the food. All the four salts were found acceptable compared to the reference salt in nine of the dishes (90% acceptability). All the nine dishes were good enough to be eaten even when darkened. In boiled plantain, salts B, C, D and R were acceptable but not salt A which has a pronounced

darkening effect.

In their raw states, all the salts were found to be acceptable but salts 'B, C and D were more preferred to salts 'R' and 'A'.

5.2 RATE OF PREVALENCE OF ANEMIA IN THE SEKYERE WEST DISTRICT AMONG CHILDREN OF 1-5 YEARS OLD

Anemia is very common among young children in many developed countries, including Ghana. It is often due to the culminating effect of a number of concurrent factors, including nutritional deficiencies, bacterial and parasitic infections. Genetic abnormalities may play a part, including sickle cell anemia.

Iron needs are high in early childhood because of the rapid increase in the total number of red blood cells and in the muscle mass, which occur with normal development. Iron deficiency anemia results when these needs are not met. This can result from one or usually more, of the following: dietary inadequacy (example, with delay introduction of iron-rich foods, overemphasis on cow's milk, which is poor in iron in some communities); suboptimal of liver and bone marrow stores (example, twins, prematures, babies of severely iron deficient mothers); or poor absorption resulting from malnutrition, together with limited bioavailability of iron in the foods

consumed.

The results revealed very high prevalence of anemia (71%) among the children (1-5 years) in the Sekyere west district. This confirms the hospital record (MOH, Mampong, 1996) which puts anemia rate of children at 60-80%.

Anemia among children may be caused by malaria, worm infestation and poor nutrition. However, the rate of malaria prevalence found among the study children was only 5.5%. Out of this, 81.7% were found to be non-anemic, ($Hb \geq 11g/dL$) and only 18.3% were found anemic ($Hb < 11g/dL$). The low prevalence of malaria (5.5%) found among the children may be due to the fact that the study was conducted during the dry season, when all the gutters and stagnant waters that serve as breeding grounds for mosquitoes had dried up. Malaria, may not be the cause of anemia found among the children. Sickle cell test was also performed on the children to check whether or not it was the cause of anemia in the children. The results showed equal numbers of sickle cell trait among both the anemic ($Hb < 11g/dL$) and the non-anemic ($Hb \geq 11g/dL$) children. The overall number of sickle cell traits among the children was found to be 21%. This value is within the range (20-30%) of sickel cell trait among the people of Tropical Africa (Fleming, 1998). The effect of sickle cell on the rate of

anemia was statistically not significant ($p = 0.8$). As a result of this one can say that the high anemia rate was not due to sickle cell disease.

The high prevalence of anemia in the Sekyere West district may be due to one of the following: poor nutrition, poor absorption of iron and/or infection with hookworm or bilharziasis.

No test was performed to find out whether the children were infested with worms or had any other infestation. This was not part of the study design. Focus of the study was on testing the efficacy of double fortified salt without interfering with other factors.

5.3 NUTRITION AND IRON DEFICIENCY ANEMIA

Iron is essential in the production of hemoglobin, which functions in the delivery of oxygen from the lungs in the electron transport system in cells, and in the synthesis of iron containing enzymes that are required to use oxygen for the production of cellular energy (Bothwell *et al.* 1979; CEC 1993).

Anemia is usually related, at least in part to iron deficiency. The importance of iron bioavailability may be illustrated by comparing the habitual dietary iron intake of the developing countries' population with the

current recommendations for dietary iron consumption of the developed countries. In the developing countries, daily iron intake ranges from 7mg/day for children to 13 mg/day among adolescent and adult females, rising to 23 mg/day for pregnant women in the third trimester (FAO/WHO 1988). These recommendations are based on the assumption of an absorption rate of about 12% absorption from a mixed diet. In many African countries, especially Ghana where millet, sorghum and maize are staple, a large amount of iron may be derived from the staple (FAO/WHO 1988). Although individuals may thus consume at least a quantity of iron meeting recommended iron intakes, the actual percentage of iron absorbed may be very low- perhaps as low as 1-2%. Thus bioavailability, not intake is usually the limiting dietary factor.

A dietary survey conducted along side this study showed that the diets of the children in the study were mainly maize, plantain and beans. These foods (e.g plantain) are known to contain polyphenols, (beans and maize) fiber, and other iron inhibiting compounds that inhibit iron absorption from the diet thereby rendering the iron in their diet unavailable. Thus, the amount of iron in their diet may meet their recommended iron intake, but the amount being absorbed may be limiting due to the presence of iron inhibiting compounds in their diet. Hence the high rate of anemia among the

children at the baseline of study.

5.4 MEAN HEMOGLOBIN OF THE CHILDREN AFTER FOUR MONTHS OF STUDY

The mean hemoglobin value of the children on the R-labelled salt increased by only 0.1g/dL at the end of the fourth month (Fig.3) but there was no change in mean Hb values of the children on the other two salts (labelled Y&G). However, when the mean hemoglobin values of all the three salts were statistically tested by ANOVA, there was no significant difference between them, ($p = 0.4$). However, there was 27% improvement in mild anemia of those assigned on the R-labelled salt (double fortified). Forty-six (46) percent of the study children on the 'R' salt were mildly anemic at the baseline but this was found to have decreased to 19% (27%). Those on the 'Y' salt was improved by 20% while those on the 'G' salt was also found to improve by only 8%. The double fortified salt was found to perform better than the rest. The four months duration may not be enough to see any meaningful changes in mean Hb values, and this may explain why there was no significant difference between the salt types after the period of this study.

Again Hb and packed cell volume (pcv) are the last indicators of iron nutrition status to change. Transferrin, ferritin, serum iron and protophyrin will all change first before Hb changes.

The following factors may also have some influence on the results. This may include infection and infestation, and poor nutrition. Intestinal worm infestation may inhibit iron absorption in the diet. Hence the amount of iron to be absorbed from the salt by the children may also be inhibited if any of them was infested with intestinal worms.

The low increase in mean hemoglobin levels of the children on the double fortified salt, may be due to poor iron absorption by the individual as a result of iron inhibitors or lack of adequate heme iron in their diet or intestinal parasites or a combination of these factors.

Some of the children had a drop in their hemoglobin. For example, six (16%) out of the 37 children remaining on the R-labelled salt had a drastic drop in their Hb by the end of the fourth month of the study. For instance, at Adidwan, two children had their hemoglobin dropping from 11.8g/dL - 7.3g/dL and 10.7 g/dL - 8.5g/dL. There was a mean hemoglobin drop of 2.1 g/dL. The drop in the hemoglobin of these children was due to illness. Some of the children had severe malaria and gastrointestinal

problems and were admitted at the hospital. Infection increases the rate of metabolism and the rate of breakdown of the tissue. This creates the need for extra nutrients yet the patients usually lose appetite and are often fed on watery gruels low in protein and iron. This may account for the decreased in their hemoglobin.

5.5 SERUM FERRITIN CONCENTRATION OF THE CHILDREN

Serum ferritin is the most specific biochemical indicator of total body iron stores. It is a useful indicator of iron status where prevalence of iron deficiency anemia is low. However, serum apoferritin is an acute-phase reactant protein that is elevated in response to infection; thus constraining interpretation in environments where the incidence of infection is high (WHO/UNICEF/UNU 1996).

The children selected for the study had their hemoglobin levels $\geq 10\text{g/dL}$ (mild to non-anemic). Thus they were expected to have normal ferritin levels ($\geq 10\mu\text{g/L}$). The ferritin concentration of all the selected children was $10\mu\text{g/L}$. The median ferritin level of the children on the R-labelled salt was found to increase by $25.8\mu\text{g/L}$ at the end of the study. Those on the 'Y' and 'G' salts were also found to have an increase in their

median ferritin concentration by 17.8 μ g/L and 22.3 μ g/L respectively. Ferritin concentration is not normally distributed hence the median was considered as an appropriate measure to use. The median ferritin concentrations of all the children on the different salts showed an improvement as shown above. But those on the double fortified salt (R) was found to perform better than the rest.

5.6 THE RATE OF IODINE DEFICIENCY AMONG CHILDREN AGED 1-5 YEARS OLD

In 1992 Asibey-Berko found a high prevalence of iodine deficiency in the mountainous areas in Ghana (Asibey-Berko, 1992) and the hinterlands. Sekyere West district, where the research was conducted is a mountainous area, hence an iodine deficient risk zone. However, with the introduction of iodated salt, one is expected to have a lower incidence of iodine deficiency in the country.

The results showed a high rate (71.4%) of iodine deficiency among the study children in the district at the baseline (UI<10 μ g/dL). From the pie chart (fig 4), 24.6% had a severe iodine deficiency. The urinary iodine levels of these

children ranged from 0.09 to 2.5 μ g/dL. Those who were moderately deficient was 17.4%, whilst the mild deficient children was found to be 29.6% (range 2.5-4.5 μ g/dL). Percentage iodine deficiency of the children on the R-labelled salt was found to be 82%, whereas those on 'Y' salt was 72.7% and 66% of the children on the 'G' salt were found to be deficient in iodine at the baseline. The high rate of iodine deficiency in the area may be due to leaching of iodine from the soil due to the mountains in the area. Food, grown in soil, poor in iodine contains insufficient iodine to meet human needs. Moreover, the people in the area do not enjoy seafood which is known to be a rich source of iodine (Burton and Foster, 1988).

Even though, iodated salt is in use in the country, the children were deficient because the people do not patronise the iodated salt due largely to ignorance. Some salt sellers have also capitalized on the ignorance of the people and still sell uniodated salt in the district, though there is a law that forbid the sale of uniodated salt in the country, (Daily Graphic, 1996).

5.7 EFFICACY OF THE DOUBLE-FORTIFIED SALT TO PREVENT ANEMIA AND IODINE DEFICIENCY AMONG CHILDREN

Four months after the children were assigned to the various salt types their blood hemoglobin, serum ferritin and urinary iodine were assessed.

As already mentioned, the double-fortified salt contained both iron and iodine. It was therefore expected that it would be able to prevent anemia as well as iodine deficiency disorders. The hemoglobin levels of the children on the double fortified salt was expected to show some increase above those on the iodized salt alone.

From the acceptability study, salt 'A' which was suspected to contain the iron and iodine because of its brown color and reaction with some of the dishes, had a similar appearance and characteristics as salt 'R'. Therefore the salt labelled R was also suspected to be the double fortified salt. It was expected that the children on the R-labelled salt would have an improved hemoglobin levels than those on the two other salts suspected to contain only iodine. The mean hemoglobin shows that there was no significant difference between all the salts contrary to expectations. However, from tables 8 and 9, the percentage mild anemia (Hb=10g/dL but <11g/dL) reduced by 27% (from 46% to 19%) for children on the R-labelled salt.

There was also a decrease in mild anemia of 20% and 8% for the children on the Y and G labelled salts respectively. The iron status (median serum ferritin) of the children on the double fortified salt improved by 25.8 μ g/L compared to an improvement of 22.3 μ g/L and 17.8 μ g/L of those on the 'G' and the 'Y' salts respectively.

The use of iodized salt has by far proved the most successful and most widely adopted method in controlling iodine deficiency.

The double fortified salt contained both iodine and iron. As expected, at the end of the study in the fourth month, the iodine status of the children improved significantly. Only 20% of the children were still found deficient at the end of the study period, whilst 80% were found to have a normal iodine status. 9.4% and 10.9% were found to have mild and moderate deficiency respectively. None was found to have a severe iodine deficiency at the end of the fourth month of study. Those who were found deficient at the end of the study could be due to the very low level of iodine status at the baseline of study. Thus they were not able to catch up before the end of the study.

Again, the percentage iodine deficiency among the children on the R-labelled salt decreased tremendously by 67% (82 to 15%), whilst those on

the Y and G-labelled salts showed a decrease of 39.4% and 35% respectively. Despite the fact that there was no statistical difference ($p = 0.9$) between the three salt types, the R-labelled salt seemed to have performed better than the rest of the salts, in terms of its ability in reducing anemia prevalence as well as iodine deficiency, considering the short four-month duration period of the study.



5.8 AVERAGE SALT CONSUMPTION

A three-day salt consumption study was conducted at Nintin and Abountem. The average salt consumption was found to be 10.55 ± 4.83 g/day (mean \pm SD). A mean value of 11.19 ± 5.74 g/day was also recorded as an average salt consumption at Abountem. This gives an average salt consumption of the people in the Sekyere-West district as 11.1 g/day \pm 4.82. With the range of 2.8-30.g/day. The mean salt consumption in the two communities is similar to the one reported by the National IDD Survey in 1992 for rural Ghanaians 10.46 g/day (Asibey-Berko and Lartey, 1992) value does not deviate from National average salt consumption (10.5 g \pm 5.8/day), documented by Asibey-Berko and Lartey in 1992. This clearly indicates

that an amount of not less than 10mg (adult equivalent) iron may be ingested from the salt daily. This is an indication that the double fortified salt contains enough iron in preventing anemia if only the rate of absorption is not inhibited. The only problem that may render the double fortified salt ineffective may be the absence of iron absorption enhancer(s) in the diet and also when the diet is rich in iron absorption inhibitors as mentioned earlier.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6:0 CONCLUSIONS

6:1 THE ACCEPTABILITY STUDY

a) Taste:

1. All the study salts labelled A, B, C and D were found acceptable in all the ten dishes. Salt 'A' was as good as the reference (R) salt but salts B, C, and D were even better than 'R'.

b) Appearance:

2. All the salts were found acceptable in 70% (7) of the dishes. The rest of the salts were found acceptable in all the 10 dishes. Salt 'A' was acceptable in seven dishes but was not acceptable in three dishes (boiled plantain, okro soup and apem ampesi). Salt 'A' imparted a dark color to these dishes.

c) Overall Acceptability

3. All the salts were acceptable in nine dishes except boiled plantain. Apem

ampesi and okro soup was found to be acceptable despite the dark color imparted by salt 'A'. They were good enough to be eaten, despite the dark color. All the salts were acceptable in boiled plantain but salt 'A' was not acceptable.

4. Salt 'A' was found to contain iron and iodine (double fortified). It was also found to be acceptable despite the fact that its darkening effect on boiled plantain, apem ampesi and okro stew.



6:2 THE EFFICACY

1. The rate of anemia among pre-school children in the Sekyere West district was found to be very high, 71%. This confirms the hospital record at the Mampong government hospital which put anemia rate as 60-80%.
2. Malaria was not the cause of anemia among the children since the prevalence rate was found to be only 5.5%.
3. Sickle cell anemia, did not have any significant effect on the high rate of anemia among the children.
3. The salt labelled R (suspected to be the double fortified salt) was found to reduce mild anemia among the study children by 27% (46-19%), and iodine deficiency by 67% (82 -15%) at the end of the study. Salts 'Y and G' were also found to reduce mild anemia by 20% (56-36%); and 8%

(36-28%)

respectively. Iodine deficiency of those on the salt-Y was found to decrease by 39.4% whereas those on the 'G' salt also decreased by 41%. The double fortified salt, (R) by reducing anemia to a higher extent performs better, in preventing anemia than the other two salts, (G and Y).

5. The R-labelled salt, (double fortified salt), was able to reduce the anemia among the children by 27% (from 46% to 19%) after four months of study.

6. There was no statistical difference ($p=0.7$) between the mean hemoglobin of males (11.2g/dL) and the females (11.1g/dL).

At the baseline, iodine deficiency rate was found to be very high (71.4%). More than twenty-four (24.6%) percent were severely deficient (0.09-2.5 μ g/dL). Whiles 17.4% were moderately deficient. Those with mild iodine deficiency were found to be 28.6%.

At the end of the four month study period, only 20% were found to be iodine deficient, while 80% had normal levels of iodine. No one was found to be severely deficient.

7. The double fortified salt (R) is effective or efficacious in preventing iodine deficiency and anemia among children in Ghana.

6.2 RECOMMENDATIONS

From the acceptability study, salt 'A' was suspected to be the double fortified salt. Even though it imparted a dark color to okro stew, boiled plantain and Apem ampesi, it is recommended as acceptable salt in Ghana.

It is recommended that, if a further study on the double fortified salt is conducted, it should be done in two different communities: the affluent and the poor communities. This will show the actual effect of the salt. Some of the study subjects should also be dewormed to rule out any effect of intestinal worm infestation on the iron.

A further study of community acceptance of the salt is recommended, to assess the acceptability of the salt in a wider segment of the population. This may also give an indication of the marketability of the salt in Ghana.

From the results of the study, one can recommend that, the double fortified salt is effective in controlling iodine deficiency and maintaining iron nutrition status among children between one and five years old.

Students should also be sent to the University of Toronto to learn the new technology of the double fortification process

APPENDICES

Appendix 1: ACCEPTABILITY STUDY

You are receiving samples of Dishes/foods seasoned with salt to compare the acceptability of salt added, in terms of: -

1. Appearance
2. Cooking taste
3. Overall acceptability

You have been given a reference salt sample marked 'R', with which you are to compare each sample. Determine whether it is more acceptable than 'R'; comparable to 'R'; or unacceptable, then mark the amount of difference that exists.

SYSTEM OF SCORING

Scoring will be done with two methods according to tables 1 & 2.

The following 3 parameters will be used for table 1:

No difference or Equal to 'R'	= 5
More acceptable than 'R'	= 9
Unacceptable	= 1

The following parameters also will be used in table 2:

No difference or Equal to 'R'	= 5
Slightly acceptable	= 6
Moderately acceptable	= 7
Very acceptable	= 8
Extremely acceptable	= 9
Slightly unacceptable	= 4
Moderately unacceptable	= 3
Very unacceptable	= 2
Extremely unacceptable	= 1

Name/code..... date.....

Table 1

Scoring	Salt type	Taste	Appearance	Overall acceptability
1.Unacceptable	707			
5.No difference				
9.More acceptable				
1.Unacceptable	105			
5.No difference				
9.More acceptable				
5.No difference	504			
9.More acceptable				
1.Unacceptable				
5.No difference	405			
9.More acceptable				
1.Unacceptable				

Comments

.....
.....

Name/code..... date.....

Table: 2

Scoring	Salt type	Taste	Appearance	Overall acceptability
1. Extremely Unacceptable	504			
2. very Unacceptable				
3. Moderately Unacceptable				
4. Slightly Unacceptable				
5. No difference				
6. Slightly acceptable				
7. Moderately acceptable				
8. Very acceptable				
9. Extremely acceptable				
1. Extremely Unacceptable	405			
2. very Unacceptable				
3. Moderately Unacceptable				
4. Slightly Unacceptable				
5. No difference				
6. Slightly acceptable				
7. Moderately acceptable				
8. Very acceptable				
9. Extremely acceptable				

Name/code..... date.....

Table: 2

Scoring	Salt type	Taste	Appearance	Overall acceptability
1. Extremely Unacceptable	707			
2. very Unacceptable				
3. Moderately Unacceptable				
4. Slightly Unacceptable				
5. No difference				
6. Slightly acceptable				
7. Moderately acceptable				
8. Very acceptable				
9. Extremely acceptable				
1. Extremely Unacceptable	105			
2. very Unacceptable				
3. Moderately Unacceptable				
4. Slightly Unacceptable				
5. No difference				
6. Slightly acceptable				
7. Moderately acceptable				
8. Very acceptable				
9. Extremely acceptable				

Comments.....

.....
.....

APPENDIX II

FORM I: COMMUNITY SCREENING AND RECRUITMENT FORM

Form No.:

Village Name:

Village Code:

1) Date:

2) Name of Interviewer:

HOME VISITS

3) Any child-bearing women in this house?

1. Yes	2. No
--------	-------

CHILDBEAR

(If "No" Interviewer proceeds to another house)
(If "Yes" meet the women).

4) House No:

5) Name of Landlord/Landlady:.....

PRELIMINARY SCREENING

Interviewer screens the women for the following inclusion criteria.

- i) Aged 15-45 years (Child-bearing years)
- ii) Has a child aged 1-5 years
- iii) Not pregnant! Not lactating!
- iv) Cooks for her family
- v) Expects to be in the village for next 6 months.

5) To such women, read and explain the CONSENT FORM (FORM II) before inviting their informed and voluntary participation in the study.

6) **SUBJECTS' RESPONSE TO CONSENT FORM**

I) Please state "Yes" or "No" Indicating whether you are willing to take part in this study or not.

RESPONSE

NAME OF RESPONDENT

A)

1. Yes	2. No
--------	-------

B)

1. Yes	2. No
--------	-------

C)

1. Yes	2. No
--------	-------

D)

1. Yes	2. No
--------	-------

9) **FINAL SCREENING FOR RECRUITMENT**

Invite consenting wives, interested husbands and their 1-5 years old children to a community meeting place on an agreed day, date and time for Haemoglobin, blood and Urine collection.

APPENDIX III

FORM II:

CONSENT FORM

It is believed that most Anaemia and Iodine Deficiency Disorders (IDD) can be controlled if our salt contains both iron and iodine.

To help the Sekyere West district where both Anaemia and IDD cases are high, persons like yourself with mild or moderate anaemia are being invited to participate in an investigation with the aim of controlling these problems.

If you are willing to participate in this study:-

- i) You and your family will have your urine and blood checked at the start of the study to see if you are iodine deficient, anaemic or have malaria parasites.
- ii) If you are selected to take part in this study, you will receive salt for your family's daily use for three months. None of the salt is to be given out to people outside your family. You should also not exchange it for other people's salt during the three months. The salt you receive may be:-
 - a) Salt containing both Iodine and iron to control both IDD and anaemia. Once a week, you will take a capsule with this.
 - b) Special salt containing Iodine to control iodine deficiency disorders. Once a week, you will take a capsule which may contain iron.
 - c) Salt containing iodine to control iodine deficiency disorders. Once a week, you will take a capsule with this.
 - d) Plain salt (same as what you always use). Once a week, you will take a capsule with this.
- iii) We shall visit you once a week to see your progress in this program.
- iv) Previous studies have shown us how much salt the average Ghanaian consumes daily. During the 3-month period, we shall periodically measure how fast you and your family are using the salt given you.
- v) If you decide, for some reason, to drop out of the study during the 3 months, you are free to do so.
- vi) At the end of the 3 months, your urine and blood will be checked again to see if you have had any improvement in your health.
- vii) If any persons are still found after three months to be anaemic or iodine deficient, these people will be given free extra medical treatment to correct the problems.

APPENDIX IV

FORM III: WEEKLY HEALTH MONITORING FORM

Form No.:

Village:

.....

To be administered at the beginning of the study and every week of the 4-month investigatory period.

1) Interviewer's Name:..... Code No.:.....

2) Date:..... Week of study:

3) Subject's Name:..... Subject No.:..... SUBJECT

4) Age: House No:..... Age:..... AGE

5) Salt color Code: SALCOLOR

6) Do you have any health disorders now?

1. Yes	2. No
--------	-------

 HEALTH

If yes, explain

.....
.....
.....
.....

7) Any issue you want to bring to our attention in connection with the study?

1. Yes	2. No
--------	-------

 COMPLAINT

If yes, explain

.....
.....
.....
.....

8) Have you suffered any ill-health since we visited you last week?

1. Yes	2. No
--------	-------

 ILLHEALTH

If yes, explain

.....
.....
.....
.....

9) Malaria is one important cause of anaemia in Ghana. Any experience of:

- Malaria since last week?

1. Yes

2. No

MALARIA

If significant malaria (ie. MP +2 or more), obtain treatment for subject and retain in study.

- Constipation since last week?

1. Yes

2. NO

CNSTIPTN

- Diarrhoea 3 or more loose watery stools on any single day since last week?

1. Yes

2. No

DIARRHOEA

- Other Gastrointestinal complaints since last week?

1. Yes

2. No

GASTRO

Appendix v



Picture Showing one of the Study Children at one of the Blood collection Centres

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