IRON INTAKE AND ITS INVITRO AVAILABILITY TO TEENAGE GIRLS IN TWO HOSTELS.

By

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LIST OF CONTENTS

Chapter			Page No.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1. INTRODUCTION

Nutritional anaemia is a common, world wide dificiency syndrome. The prevalence of iron responsive microcytic, hypochromic anaemia various from 9 to 70 per cent of different population groups examined, the incidence is higher in less developed countries and in under privileged segments of population in the industrialised world (Chandra, 1981). Iron deficiency anaemia is a world wide nutritional problem, its prevalence being particularly high among women, pregnant women and children. Anaemia is an important public health problem in many developing countries including India (ICMR, 1982).

It is estimated that 40-60 per cent preschool children, 25-30 per cent women of child bearing age and almost 50 per cent pregnant women in the third trimester of pregnancy suffer from anaemia in India (ICMR, 1982). Of one billion women in their reproductive years, it appears that atleast half of the non-pregnant and nearly two-thirds of pregnant women have anaemia. This makes a total of some 260 million anaemic women in the developing world alone (WHO, 1979). There is a high prevalence of iron deficiency in adult women of the child bearing age. Most of this iron deficiency is nutritional in the sense that the diet does not contain sufficient quantities of available iron to replace physiologic losses associated with menstruation and pregnancy (Cook, 1977).

There is widespread prevalance of anaemia in children and pregnant women of the low-economic groups. Nearly 50 per cent of pregnant women in these groups have haemoglobin levels below 10g per 100ml and as much as 20 per cent of maternal deaths may be attributable to anaemia (NIN, 1977).

Apart from the reduced haemoglobin concentration in blood which forms the basis of defining anaemia, iron deficiency is reported to lead to a number of deleterious effects of great socio-economic relevance like i) impaired work capacity ii) Poor obstetric outcome iii) poor learning ability and iv) impaired resistance to infection. Cellular immunity has been shown to be impaired in iron deficiency in patients and also in experimental animals. The manifestations include i) decreased skin reactivity to intra-dermal injection of antigens; ii) impaired bacterial killing by neutrophils, to reduce the dye nitro-blue tetrazolium (NBT), iii) fewer T cells and decreased lymphocyte proliferation in response to mitogens.

Humoral immunity, in contrast to cellular immunity is generally found to be unimpaired. Iron deficient patients have normal or elevated concentration of immunoglobulins in the plasma with normal antibody response to immunization and normal opsonic activity (ICMR, 1982).

Clinical manifestations of iron deficiency include, fatiguability, weakness, shortness of breath, palpitation, tachycardia and vertigo.

Iron deficiency, even subclinical depletion of iron stores, is causally associated with many tissue changes. Flattening and spooning of nails, gastric mucosal atrophy, keratinization of buccal mucous membrane, smooth tongue with flattened papillae, shortened inestinal villus height, and atrophy of lymphoid organs have been documented in iron-deficient individuals (Chandra, 1981).

Groups at risk due to anaemia are i) children and adolescents, especially at times of groth spurts, when blood volume is increasing, ii) girls and women, due to menstruation especially at adolescence when growth is coincident, iii) women undergoing a succession of pregnancies (Rankin et al., 1976). The child is often plump and appears well nourished but is pale and even bloated in appearance (Food and Nutrition Board, 1980). Anaemia among infants and children may lead to impaired psychomotor development and learning ability and such defects are corrected when anaemia is treated with iron (ICMR, 1982). Severe anaemia during pregnancy is associated with an increased risk of maternal and fetal morbidity and mortality. Even mild anaemia has been show to be associated with an increased risk of premature delivery, low birth weight, placental hypertrophy and reduced estriol excretion. Zinc deficiency, which may accompany iron deficiency, can lead to growth retardation in adolescents and may also contribute to the production of some of the classical signs of iron deficiency such as koilonychia and plummervision sydrome (INACG, 1977).

The recommended dietary allowances of iron for adolescent girls is 35 mg. (Food and Nutrition Board, 1980). The mean energy intake by the teenage girls was 2088 k.cals. The iron intake ranged from 6 to 28 mg daily, mean 14.9 mg daily. It is apparent that in only 17 per cent of the girls the iron intake was greater than the recommended allowance (18mg). In 10 per cent it was less than 10 mg daily (Elsborg et al., 1979).

Iron deficiency is commonly the result of inadequate intake of iron, failure of absorption and excessive loss of iron from the body as a result of physiological and pathological process (Apte and Venkatachalam, 1963). In the etiology of this disease, dietary factors are considered to play an important role. Poor absorption of iron from diets predominantly based on plant foods is considered to be an important limiting factor in fulfilling iron needs of population subsisting on such diets (Narasinga Rao, 1971). Iron deficiency anaemia is widespread in India inspite of seemingly adequate intakh of dietary iron. One of the important factor may be poor absorption of dietary iron (NIN, 1975).

Absorption of iron is favoured by certain coincident nutrients. Ascorbic acid, which is a reducing agent assisted by coverting iron in the iron III (ferric) form to the iron II (ferrous) form necessary for absorption (Rankin et al., 1976). Carbohydrate and fat had little influence where as egg albumin had a significant inhibitary effect on the absorption of non-heme iron (Monsen et al., 1979). It has been determined that several chemical factors such as valence, solubility and ability to form complexes will influence the biological availability of iron from foods. Solubility may be a pre-requisite to iron absorption, as recently demonstrated by Mot zok et al., (1978) who confirmed the direct correlation of availability with solubility of elemental iron sources (ken lee et al., 1980). Hallberg et al.(1982) found a reduction in iron absorption (62 per cent) while serving tea or (35 per cent) when serving coffee. Pure alcohol and wine increased only slightly the percentage absorbed. Orange juice increased the iron absorption (85 per cent) wine often has a high iron content, which increases significantly the amount of iron absorbed (three times). Milk and beer have no significant effect. Coca-cola increased only slightly the absorption (Hallberg et al., 1982)

Many adolescent girls, even today, select a poor diet, to indulge the whims of a freakish appetite or to maintain ill-advised reduction regimens, with resultant anaemia. Thus it is necessary to continually emphasize the fact that during adolescence there is an accelerated demand for iron to satisfy the still increasing blood volume as well as compensate for losses through menstruation. Normal mixed human diets of good quality contain approximately 12-15 mg of iron of which slightly more than 10 mg is absorbed. This amount is adequate for adult males, but it is inadequate for adolescent girls or women on diets of a less than 10 per cent calorie content from animal foods (Passmore et al., 1974).

Several invivo methods for the determination of bioavailability of food iron have been proposed. However, these invivo methods are accurate they are time consuming and expensive for screening large number of food materials. Because of this limitation invitro methods have been proposed. In the present study, an attempt has been made to determine the bioavailability of iron from foods consumed by the teenage girls in a hostel using the invitro method described by Narasinga Rao and Prabhavathi (1978). The food intake was assessed by a three day food weighment survey. Fourty girls of age group between 13 to 19 years from the hostels of Sri Avinashilingam High School and College were selected at random for this study. The enhancing effect of ascorbic acid on iron availability and the inhibiting effect of tannic acid on iron availability were also taken into consideration for the present study.

Such an invitro method for determining the bioavailability of food iron is desirable in the context of indentifying the causes of anaemia and preventing it. This method can also be applied to suggest improvements in the diet to enhance iron absorption from diets and iron sources that are intended for use in the control and prevention of iron deficiency anaemia.

II REVIEW OF LITERATURE

The literature pertaining to this study "Iron intake and its *invitro* availability to teenage girls in a hostel" is discussed under the following heads.

1. Iron deficiency anaemia and its causes
2. Population susceptible to iron deficiency anaemia.
3. Iron requirements.
4. Absorption of iron from foods.
   a. Iron absorption from a rice based meal.
   b. Iron absorption from wheat.
   c. Iron absorption from ragi (*Eleusine coracana*) and sorghum (*Sorghum vulgare*).
   d. Iron absorption from maize (*Zea mays*) and sorghum (*Sorghum vulgare*) beer.
   e. Iron absorption from Amaranth.
   f. Factors increasing iron absorption.
   g. Inhibitors of iron absorption.
5. Bioavailability of food iron.
6. Assessment of bioavailability of iron in the whole diet.
   a. *Invivo* methods
   b. *Invitro* methods.

1. Iron deficiency anaemia and its causes

Iron deficiency must be defined as a reduction of total body iron below normal levels. Arbitrarily iron deficiency may be classified as mild, moderate and severe. Mild iron defeciency exists when the total body iron is reduced but there is sufficient iron to provide a normal mass of haemoglobin. The term "iron deficiency anaemia" is properly used to describe moderate and severe iron deficiency because there is insufficient iron to make normal quantities of haemoglobin (Conrad, 1967).

In the developing countries, apart from increased requirements the commonest causes of iron deficiency are poor availability for dietary iron and increased iron losses due to parasitic infestations, especially hook worm. In developed countries iron deficiency is less common but when it occurs it may be due to inadequate dietary iron intake associated with reduced energy intake or to the presence of some pathological state interfering with iron absorption or producing increased iron losses. Cook and Finch (1972) suggested that iron deficiency can be identified at three levels;

i) iron depletion ii) iron deficient erythropoiesis and iii) iron deficiency anaemia.

Factors related to iron deficiency are

1. Haemorrhage

   a. Benign and malignant lesions

   b. Trauma

   c. Intestinal parasites

   d. Coagulation defects.

2. Malabsorptive syndrome

   a. Gastric

   b. Small intestinal

3. Dietary Factors

   a. Iron content of diet

   b. Composition of diet

   c. Protein deprivation.

4. Inflamation and infection

   a. Growth

   b. Pregnancy.

Severe iron deficiency affects the processes of the central nervous system, which in turn affect the infantile growth period, susceptible to abnormalities which might not be remediable by subsequent provision of adequate iron intake (Leibel, 1977). Iron deficiency is common in tropical countries and one hypothesis to explain this high prevalence was excessive loss of iron in sweat (Mitchell et al., 1949 and Apte et al., 1963). Anaemias in general are related to low work efficiency (Cifuentes and Viteri, 1972; Davies and Van Haaren, 1973). The amount of body iron may affect the work performance even in the absence of anaemia (Finch et al., 1979)

Iron deficiency, both in man and animal has been shown to produce a number of alterations in immunological systems such as impairement of lymphocyte transformations (Srikantia et al., 1976 and Johnson et al., 1972), decreased production of migration inhibition factor and impaired cutaneous delayed hypersensitivity (Chandra, 1976). Neutrophil function may also be deranged although studies by Kulapongs et al., (1974), showed normal leucocyte function.

Severe anaemia during pregnancy is associated with an increased risk of maternal and fetal morbidity and mortality. Even mild anaemia has been shown to be associated with an increased risk of premature delivery, low birth weight, placental hypertrophy and reduced estriol excretion. It is probable that iron deficiency may affect many bodily functions. Abnormalities such as decreased gastric juice secretion, reduced activity of intestinal cell enzymes, and subcellular structural abnormalities including mitochondrial enlargement have been documented in iron deficiency. Iron deficiency, can lead to growth retardation in adolescents and may also contribute to the production of some of the classical signs of iron deficiency such as Koilonychia and Plummer-vision syndrome (INACG,1977).

Iron deficiency anaemia make children more prone to respiratory infections and can also lead to behavioral changes and reduced and learning ability. Improvement of subnormal mental performance in iron deficient infants and children has been reported, following the administration of iron (Garn and Smith, 1973 ; Webb and Oski, 1973).

Iron deficiency anaemia is a medical and public health problem of primary importance, causing few deaths but contributing seriously to the weakness, ill health and substandard performance of millions of people (Passmore et al., 1974)

<u>Population susceptible to iron deficiency anaemia</u>

Iron deficiency is most likely to occur when iron requirements are greatest, that is, during pregnancy, childhood and the reproductive age. An inadequate dietary intake of iron by growing children, by adolescent girls, or by women, especially during pregnancy and in lactation, will produce nutritional anaemia, characterized by a decrease in the amount of haemoglobin and by small pale-red blood cells, depleted iron stores, and a plasma iron content of less than 40 mg. per 100 ml. The number of red blood cells may also be reduced, but not as markedly as the haemoglobin content (Passmore *et al*., 1974). If the pregnant woman has an insufficient intake of iron, the newborn infant, inturn, will have a relatively low store of iron, causing anaemia to develop early in the first year of life. Anaemia during infancy, a frequent phenomenon, is closely related to the body stores of iron at birth. It is especially common in premature infants and twins, because in such circumstances the body reserves of iron cannot be built upto desirable levels (ICMR, 1982).

3. <u>Iron requirements</u>

Dietary iron is required for replacement of small daily losses in all individuals, an expanding blood volume and increasing amounts of haemoglobin in growing children, replacement of varying losses through menstruation, development of the foetus and to avoid anaemia in the pregnant or lactating woman, and a reserve of iron which is available when blood loss may occur from any cause whatsoever (Proudfit *et al*., 1963). Only very small amounts of iron are lost from the body, mostly in the cells, or shed from the skin and the epithelial surfaces lining the alimentary and urinary tracts (Passmore *et al*., 1974). The amount of iron which must be ingested to achieve the required daily absorption depends to a large extent on the type of diet (INACG, 1977). Iron requirements are influenced by the availability of iron present in foods. Iron present in cereals, legumes and green leafy vegetables is available to a lesser extent (due to the presence of phytates and oxalates) than that present in eggs, meat and fish (Swaminathan, 1976).

The iron requirements of an adult woman is much higher because of the loss of 2mg. of iron per day in the menstrual blood. In the case of infants, the iron requirements should not only compensate for basal iron losses, but also provide for an increase in haemoglobin and in iron content of body tissues associated with growth. During adolescence there is an accelerated demand for iron to satisfy the still increasing blood volume as compensate for losses through menstruation (Passmore et al., 1974). After the menarche there occurs a great increase in the requirement, partly because of loss through the menstrual bleeding and partly as a result of rapid growth during the early phase of puberty. The investigations show that the diet taken by teenage girls is sufficient with respect to iron ($\geq$ 10 mg daily) in the majority upto the menarche, but after the onset of puberty only about 17 per cent receive iron enough in relation to the recommended daily allowances to ($\geq$ 18 mg daily). The iron intake was definitely correlated to the energy intake (Elsberg et al., 1979).

The iron requirements as stipulated by Indian council of Medical Research (ICMR, 1981) for adult men, women and lactating women is 0.72, 1.6 and 3.4 mg per day respectively while it is 1.0 mg per kilogram body weight of infants and 0.65 mg per day for children.

4. Absorption of iron from foods

4. Absorption of iron from foods

The most important constraint in meeting iron requirements through dietary means is the limited absorption of dietary iron. Iron absorption for different diets varied from 7 to 20 per cent of the iron they contained, with a mean of 13 per cent. Considering the fact that Indian diets are fairly rich in iron, containing as they do, about 20 to 30 mg of iron, this level of absorption should not lead to any iron deficiency in the population (Narasinga Rao, 1971). Absorption of iron can take place from the stomach and throughout the whole of the small intestine, however, the greatest absorption occurs in the upper part of the small intestine. Only 10 per cent of the iron present in cereals, vegetables, and pulses, excluding soybeans, is absorbed (Passmore *et al*., 1974). The iron status of the individual, his erythropoietic rate, and other factors within the body regulate the process of iron absorption, particularly at the level of transfer across the serosal surface (Munro, 1977). There is evidence that absorption responds to increased demand for iron by the body such as when new red blood cells are being formed in the bone marrow of growing children and pregnant women (Rankin *et al*., 1976).

a) Iron absorption from a rice based meal

Studies regarding the absorption of iron from rice was done using extrinsically added radioactive iron to a standard meal. In normal subjects the mean absorption of iron from the meal was 3.3 per cent while in anaemic subjects, the mean absorption was 12.6 per cent (NIN, 1973). Iron absorption from rice based diets was determined in apparently well-nourished subjects by whole body counting. The mean absorption of iron in these subjects was found to be 5.5 per cent (NIN, 1975).

b) Iron absorption from wheat

It was reported that 8-10 per cent of iron present in wheat is absorbed by normal adult males. These studies were done using radioactive iron, employing the standard meal technique. Though many studies on iron absorption have been done using this method, its validity has not yet been fully established. A study was therefore, undertaken by NIN (1972) to compare absorption observed by this procedure with absorption observed by use of biosynthetically labelled wheat iron. Iron absorption from meals based entirely on wheat as measured by this method ranged from 1.9-2.1 per cent. These values are significantly lower than those obtained with the rice based meal. In a mixed cereal diet containing both wheat and rice the absorption was 3.3 per cent a value intermediate to those observed with diets based upon rice or wheat alone (Narasinga Rao, 1971). The absorption of iron is enhanced by the presence of lysine and histidine (Campen *et al.*, 1969).

c) Iron absorption from ragi (*Elusine Coracana*) and sorghum (*Sorghum vulgare*)

Sorghum and ragi constitute the staple food of a sizable segment of population in South India. Studies using extrinsic tag method indicate that the mean iron absorption from ragi was 2.2 per cent and that from sorghum was 1.4 per cent (NIN, 1975).

d) Iron absorption from maize (*Zea mays*) and sorghum (*Sorghum vulgare*) beer.

Iron absorption from maize and sorghum beer was more than twelve fold greater than from a gruel made from the constituent used to prepare the beer. Three factors are responsible for the enhanced iron absorption from maize and sorghum beer. These include the removal of solids during fermentation and the presence of ethanol and of lactic acid in the final brew (Derman *et al.*, 1980).

e) Iron absorption from amaranth

Green leafy vegetables are good sources of iron but the availability of iron from this source has not been well investigated. The mean iron absorption from biosynthetically labelled 59 Fe amaranth which constitute a part of a meal consisting of wheat flour chappathies and potatoes was 2.1 per cent. This value, however, may not reflect iron absorption from amaranth because the presence of wheat containing phytates may have reduced iron absorption from amaranth (NIN, 1972).

f) Factors increasing iron absorption

Iron transport across the intestinal mucosa begins with absorption of ionic iron in the lumen of the small intestine to receptors in the brush border of the mucosal cells (Linder *et al*., 1977). There are two kinds of iron compounds in the diet with respect to the mechanism of absorption heme iron (derived from haemoglobin and myoglobin) and non-heme iron (derived mainly from cereals, fruits and vegetables). The absorption of these two kinds of iron is influenced differently by dietary factors. Heme iron forms a relatively minor part of iron intake. Non-heme iron is the main source of dietary iron (Hallberg, 1981). A large number of factors are known to increase or facilitate iron absorption (Apte, 1966).

1. Anaemia

a. Iron deficiency

b. Hemolytic

c. Acute blood loss

d. Sideroblastic refractory

2. Hypoxia

3. Erythropoietin administration

4. Cobalt administration

5. Liver regeneration (cirrhosis)

6. Portacaval shunts

7. Pancreatic insufficiency

8. Pyridoxine deficiency

9. Pregnancy

10. Miscellaneous factors

a. Ascorbic acid

b. Succinic acid

c. Sorbitol

d. Ethanol

e. Decreased p^H

The main factors that influence the absorption of iron from the diet are a) the amounts of heme and non-heme iron, b) the content of the dietary factors influencing iron bioavailability, and c) the iron status of the subjects. Heme and non-heme iron are affected differently, not only by dietary factors but also by the subjects iron status (Hallberg, 1981). The bio-availability of iron in meals containing meat is about 25 per cent and the bioavailability of heme iron given without meat or liver has a maximum absorption of about 10 per cent decreasing with increasing dose to a few per cent (Hallberg, 1979). Studies employing radio iron have shown that absorption from vegetable and cereal foods is less than 5 per cent as compared with 15-20 per cent absorption from animal sources (Layrisse et al., 1973). Studies indicate that a certain amount of animal feed should be present in the diet to enhance iron absorption from vegetable feed (Cook et al., 1971). Definite increase in the iron absorption is observed with increasing intake of dietary calcium (Apte et al., 1964).

The composition of the diet and the levels of different nutrients there in are important factors influencing iron absorption (Apte et al., 1965). The absorption of non-heme iron is markedly influenced by the iron status of the subject-more iron is absorbed by the iron dificient and less by the iron-replete subject (Hallberg, 1981).

The bioavailability of dietary iron increases during pregnancy and is roughly, parallel to the increased iron requirements (Svanberg et al., 1975). In idiopathic hemochromatosis, the absorption of food iron is markedly increased in relation to the size of the iron stores (Bezwoda et al., 1976).

In normal subjects the absorption of iron in rice and spinach was only about 1-2 per cent but in animal foods such as beef and veal liver it amounted to 10-20 per cent (Layrisse et al., 1971). The absorption of iron is enhanced by the presence of lysine and histidine. Lysine is the first limiting amino acid in wheat and fortification of wheat with this amino acid has been suggested as a method of improving, the quality of wheat protein (NIN, 1972).

Products of protein digestion favour the absorption of iron. This may be due to the reducing properties of the sulphydryl groups (Rankin et al., 1976).

Carbohydrate and fat had little influence on iron absorption (Monsen *et al.*, 1979). The presence in the intestine of the food components such as fructose, ascorbic acid and cysteine increase the amount of iron absorbed (Lock *et al.*, 1980). An enhancing effect of meat and fish was first reported by Layrisse *et al.*, (1968).

The p^H influenced ionization and valence changes of elemental iron (EI), ferrous sulphate (FS), ferric orthophosphate (FOP) and sodium ferric EDTA (SFEDTA), thus increase the absorption of iron. It has been determined that several chemical factors, such as valence, solubility and ability to form complexes, will influence the biological availability of iron from foods (Kan Lee *et al.*, 1980). Iron in milled, polished rice, is about four times better absorbed than is iron in unmilled rice (Bjorn-Rasmussen *et al.*, 1973).

Moore and Dubach (1951) and Pirzio-Biroli *et al.* (1958) found that ascorbic acid in doses of 250 mg to 500 mg given in crystalline form or as fruit juice increased assimilation of food iron. The increase was found to be greater in patients feed iron, suffering from iron deficiency anaemia than in normal subjects. This effect of large amount of vitamin-C has been attributed to the easy liberation and reduction of ferric iron to ferrous state in the more acidic medium. In both male and female adults supplementation of 100 mg of vitamin-C in crystalline form along with the feed, brought about a significant reduction in iron loss in stools (Apte *et al.*, 1968).

A drink can increase the iron absorption from a meal not only by increasing the bioavailability but also by increasing the total iron content of the meal (example wine). Orange juice increased the iron absorption (8.5 per cent). Pure alcohol and wine increased only slightly the per-centage absorbed. Wine often has a high iron content, which increases significantly the amount of iron absorbed (three times). Milk and beer have no significant effect. Coca-cola increased only slightly the absorption. The absorption promoting effect of alcohol on ferric iron salts was shown to be probably caused by a stimulating action of alcohol on the secretion of gastric hydrochloric acid (Hall berg *et al.*, 1982). When ascorbic acid was added during cooking there was a three fold increase in the absorption of both intrinsic iron and supplementary iron when a sufficient quantity (40 mg) was present (Sayers et al., 1974)

Orange juice containing 70 mg of ascorbic acid increased iron absorption from a breakfast meal 2.5 times (Rossander et al., 1979). The addition of cauliflower, which also contains about 70 mg of ascorbic acid, to a vegetarian meal increased the absorption of non-heme iron three times, from 0.32 to 0.98 mg. Similar effects were observed in studies on meals conta-ining rice, maize, wheat or soy (Sayers et al., 1974). In one study on corn flour meals, a six-fold increase in absorption was obtained both with 70 mg of ascorbic acid and with papaya, which contains about the same amount of ascorbic acid (Layrisse et al., 1974). When ascorbic acid is added to arridge it potentiates the absorption of iron present in maize meal (Sayers et al., 1974).

Inclusion of known absorption promoters like ascorbic acid and meat extract increased ionizable iron at p^{H} 7.5 (NIN, 1977). It was shown that ascorbic acid or orange juice with a high content of ascorbic acid markedly increased food iron absorption. The overall effect of ascorbic acid was as a reducing agent at low p^H also in promoting oxidation at higher p^H values, thus tending to increase ionization (Nojeim *et al.*, 1981).

g. Inhibitors of iron absorption

Iron absorption in humans is considered to be adversely affected by the presence of the following factors. (Nutrition Society of India, 1966).

1. Iron overload
2. Aplastic anaemia
3. Transfusional polycythemia
4. Generalized malabsorption states.
5. Miscellaneous factors
a. Phytates
b. Deferoxamine
c. Phosphates
d. Pancreatin
e. Clay.

Recent studies on single foods have shown that milling of cereals greatly affects their iron bioavailability (Bjorn-Rasmussen *et al.*, 1973). The amounts of iron solubilished when the foods were mixed with bran, oats or egg (known to reduce the invivo absorption of iron from foods) were less than calculated from the sum of each absorption (Lock *et al.*, 1980). Definite decrease in the iron absorption is observed with increased intake of dietary calcium due to the formation of insoluble salts by reaction with oxalate and phytate ions and with the free fatty acids which are present in the intestines (Cook *et al.*, 1964).

Several studies have shown that sodium phytate decreases iron absorption in man (Mc cance *et al.*, 1973). The lower fraction of iron absorbed from brown bread compared with white has been attributed to the high content of iron phytates in bran (Hallberg, 1981). Most of the phytates however, was brokendown during leavening and baking of the bread, with a corresponding increase in content of phosphate. It is possible as has been suggested that the inhibitory effect of bran is partly due to its content of fiber components (Bjorn-Rasmussen, 1974).

Eggs have been reported to decrease the absorption of iron from a breakfast meal. Egg yolk was found to decrease the absorption of iron from an inorganic iron salt given to rats (Callender *et al.*, 1970). Absorption of iron as percentage of intake was found to be 18.9 ± 1.08 (without egg), 14.5 ± 0.94 (with egg), 26.4 ± 2.08 (with egg and ascorbic acid as a single dose) and 32.3 ± 0.65 (with egg and asc-orbic acid supplemented individual doses). These studies showed a reduction in the percentage absorption of iron when the egg was included in the diet (Rathee *et al.*, 1980).

Milk has been found to decrease iron absorption from meals with a low bioavailability. Oxalate will decrease the iron absorption. For cystein, divergent results have been obtained. Some studies showed no effect (Bjorn-Rasmussen *et al.*, 1970), whereas other studies have shown a marked absorption promoting effect (Martinez-Torres et al., 1970).

Maize eaten with liver reduced the absorption of liver iron, indicating an inhibiting effect of maize on the absorption of non-heme liver iron (Hallberg, 1981). Egg albumen had a significant inhibitary effect on the absorption of non-heme iron (Hansen et al.,1979). Ionizable iron at P^{H} 7.5 decreased in the presence of phytate and tannins (Rao et al., 1978). EDTA is the most widely used synthetic chelate of polyvalent cations incorporated into the diet substantially reduces the absorption of food iron (Cook et al., 1976).

Recently it was reported that tea markedly reduced the absorption of nin-heme iron from foods. Conrad et al.(1962) attributed this effect to the formation of iron-tannate complexes. Tannates are also present in coffee. The inhibitary effect of coffee is due to tannates. The seed cost of legumes wa s the source of tannin which reduced the ionizable iron content of the whole grain. Other sources of tannin in the diet are tamarind and tea. Tea when added to a meal was found to reduce the lionizable iron of that meal. Similarly the use of tamarind extract as a source of acid in our practice may also reduce the ionizable iron (NIN, 1979). It might be expected that coffee should enhance the absorption of nonheme iron by stimulating the secretion of gastric juice. It is possible that the inhibitary effect of tannins or other compounds present in coffee is more marked then the stimulating effect mediated by an increased secretion of gastric juice (Hallberg et al., 1982). The absorption of iron from bread was reduced to one third and from a vegetable soup to one fourth when served with tea compared with water (Disler et al., 1975). In a western type breakfast, the absorption was reduced about 60 per cent by tea (Rossander et al., 1979). This effect has been ascribed to the formation of iron-tannate complexes (Conrad et al., 1962). It has also been suggested that tannins may be partly responsible for the low bioavailability of iron in many vegetable foods (Disler et al., 1975).

5. Bioavailability of food iron

The bioavailability of iron in a food is not the sum of the absorption of iron from the single foods contained in a meal, but rather a net effect of all food items, and their constituents increasing or decreasing non-heme iron absorption. The biavailability of iron in various foods was expressed as the ratio of food iron absorption to the absorption from a reference dose (Hallberg, 1982). Current knowledge about food iron absorption implies that the bioavailability of iron in a diet depends not only on its content of heme and non-heme iron, but to a large extent on the balance between factors that stimulate (ascorbic acid and meat extract) and inhibit (phytate and tannins) the absorption of iron. The amount of iron potentially available from diets depends not only on the quantity and quality (Nature) of iron present, but also on the other dietary components, which may alter significantly the utilization of iron (Hallberg et al., 1974 ; Layrisse et al., 1974).

In normal persons absorption of iron in rice and spinach was only about 1-2 per cent but in animal foods such as beef and veal liver it amounted to 10-20 per cent (Layrisse et al., 1971). In rice based diets 3-8.5 per cent (NIN, 1972) from wheat based meals 2 per cent, (NIN, 1972) from ragi 2.2 per cent and from sorghum 1.4 per cent (NIN, 1975).

Although the concentration of iron in cereals and pulses is low, they are the most important sources of dietary iron as a substantial amount of dietary iron is derived from them. But the iron present in cereals and pulses is available to a less extent as cereals are rich in phytate, a factor known to have an adverse effect on iron availability. Basic information on the availability of iron from single food is essential to obtain. Such information in the first instance provides the basis for understanding the availability of iron from various diets. In the second instance the information obtained can be used to improve the availability of iron from diets.

6. Assessment of bioavailability of iron in the whole diet

a. Invivo methods

The total daily absor-ption of dietary iron has also been measured with the extrinsic tag method, labelling both heme and non-heme iron with two different radio iron isotopes. The mean total daily absorption was 1.0 mg (Bjorn-Rasmussen *et al.*, 1974). Recent studies have shown that the absorption of iron is lower from homogenized than from identical non-homogenized meals (Hallberg *et al.*, 1977).

In a recent study in Sweden (Olsson *et al.*, 1978) in healthy men in whom iron-deficiency anaemia was induced by repeated phlebotomies, the rate of haemoglobin recovery indicated an average total absorption of iron from the diet of 3.8 mg per day.

Since the basal losses of iron are about 1.0 mg per day in men, the total amount of iron absorbed during this repletion period was 4.8 mg per day. The average bioavailability of dietary iron for non-anaemic subjects, with no iron stores would be 2.4 mg per day (Hall berg, 1981).

B. Invitro methods

The invitro tests for iron availability involve the determination of ionizable iron in foods and quantification of the iron liberated by pepsin-hydrochloric acid treatment. Pepsin-hydrochloric acid treatment has been done in determining the ionizable iron content of foods so as to stimulate conditions of gastric digestion. Studies were carried out by Narasinga Rao and Prabhavathi (1977) to determine whether the release of ionizable iron from foods subjected to treatment with acid pepsin at P^H 1.35 and subsequent adjustment of P^H to 7.5 stimulating some what the conditions of gastric digestion and duodenal P^H. Values obtained by these methods well correlated with invivo absorption values obtained in humans and that factors which affect invivo absorption also affected invitro absorption in a similar manner.

III. EXPERIMENTAL PROCEDURE

This investigation was designed to study "Iron intake and its invitro availability to teenage girls in a hostel".

The procedure consisted of the following steps.

A. Selection and grouping of the teenage girls.

B. Conducting a food weighment survey.

C. Determination of bioavailability or iron.

A. Selection and grouping of the teenage girls

Many teenage adolescent girls, even today, select a poor diet, to indulge the whims of a freakish appetite or to maintain ill-advised reduction regimens, with resultant anaemia. Thus it is necessary to continually emphasize the fact that during adolescence there is an accelerated demand for iron to satisfy the still increasing blood volume as well as to compensate for losses through menstruation (Passmore *et al.*, 1974). Hence an attempt was made to study the iron intake of adolescent girls in this study. Twenty girls were selected at random from the school hostel and an equal number of girls from the college hostel of the

Sri Avinashilingam Institutions for the study. The age range of the selected students was 13-19 years.

B. Conducting a food weighment survey

Food weighment method is a positive step in increasing the efficiency of eating habits by finding out the needs (Ramasastri *et al*., 1973). A three day weighment survey would be as efficient as that of the seven day weighment, if the dietary variation in the day to day life is not large. Since the dietary pattern in India doesnot vary greatly from day to day a three day weighment was done. Five subjects from each age group were selected randomly and a three day food weighment survey was carried out. All the cooked food items were first weighed and given to the subjects. After the food was consumed, the quantity of each food items which remained unconsumed was weighed to record the plate wastes. From the above data the quantity of the food consumed by the individual was obtained. Ten per cent of the food items actually consumed in a day by the selected subjects were collected to determine the iron intake in a day. Ten per cent of each of the individual meals consumed for breakfast, lunch and dinner was also collected separately for the analysis. Thus the effort was aimed towards quantifying the diets consumed by the targets and there by finding out the amount of total, ionizable and solubbe iron available in their diets.

C. Determination of bioavailability of iron

1) Estimation of iron in a day's food

Ten per cent of the diet consumed by each subject during each of the meal time that is, breakfast, lunch, tea and dinner was collected, pooled together and homogenised in a blender. About 5.0g of the homogenised cooked food was mixed with 50ml of pepsin-hydrochloric acid solution (0.5% pepsin in 0.1 N HCL). The P^H of the mixture was adjusted to 7.5 with sodium hydroxide and the mixture was incubated at 37°C for 90 minutes. The contents were then transferred into test tubes and boiled for about 10 to 15 minutes in a water bath. The test tubes were kept immediately in ice for about 5 minutes till suspended particles settled. The contents were thereafter centrifuged at 3000 r.p.m. for 45 minutes and the clear supernatant fluid was filered through whatman No.44 filter paper. The filtrate was used for the deermination of solubbe and ionizable iron.

Ionizable iron

Ionizable iron was determined by dipyridyl method as given in Appendix-I, Iron in the filtrate obtained after incubating foods at p^H 7.5 is the ionizable iron.

Soluble iron

Soluble iron is the amount of Fe^{2+} and Fe^{3+} ioris complexed to other biomolecules. This was determined by the method of Tennet and Greenman (1969) as shown in Appendix-I, after digesting the filtrate with pottasium permanaganate and decolourising the same with vitamin-C and filtered. The iron in the filtrate was determined by the $\alpha\alpha$-dipyridyl method .

Total iron

The food was dry ashed as described by Association of official Agricultural chemists (1975). Total iron in the mineral solution was estimated by the thiocynate method of Wong as given in Appendix-II.

Ionizable iron at p^H 7.5 (Jejunal and duodenal p^H) was taken as a measure of iron available since the site of absorption of iron is the duodenum. The values are expressed as ionizable iron per 100 units of total iron in a food. Absolute available iron was calculated as follows.

$$\text{Absolute available iron} = \frac{\text{Per cent ionizable iron at } p^H \text{ 7.5}}{100} \times \text{Total iron}$$

ii) Estimation of iron in individual foods

Ten per cent of each of the meals consumed in a day was collected separately and homogenised in a blender. About 5.0 g of the homogenised cooked food was extracted with pepsin-hydrochloric acid and the filtrate was used for the determination of soluble and ionizable iron as described earlier.

iii) Effect of lime juice on iron availability

The ascorbic acid content of lime juice was determined by dye method as given in Appendix-III and the effect of lime juice on ionizable iron was determined by mixing it with breakfast. Ionizable iron was determined as described earlier in 5.0 g of the homogenised breakfast to which the lime juice was also added.

iv) Effect of tea on iron availability

Effect of tea on ionizable iron of the food was determined by adding tea solution to the breakfast. The tannin content of the added tea solution was determined by the method of Association of Official Agricultural Chemists (1965) as given in Appendix-IV

About 5.0 g of the homigenised breakfast was utilised for this purpose and the ionizable iron was determined by the above said method.

IV. RESULTS AND DISCUSSION

Food weighment survey was carried out on three consecutive days among the 40 girls selected randomly from both the school and college hostels of Sri Avinashilingam Institutions and the total iron content of the food they consumed was determined by the thiocyanate method of Wong. The bioavailability of non-heme iron from various foods consumed by teenage girls of both hostels was determined by an invitro techniques. Studies of Narasinga Rao and Prabhavathi (1978) have shown that per cent ionizable iron at p^H 7.5 correlate highly with per cent iron absorption from the same diets as measured by whole body counter in adult males. Hence in the present investigation the ionizable iron at p^H 7.5 was determined by the invitro technique of Narasinga Rao and Prabhavathi (1978) to measure the bioavailability of non-heme iron in foods.

The invitro bioavailability of iron from individual meals and the effect of tea and lime juice on iron absorption in breakfast have also been studied.

The iron intake of teenage girls is presented in Table-I and Figure-I and the individual values are given in Appendix-V.

TABLE-I

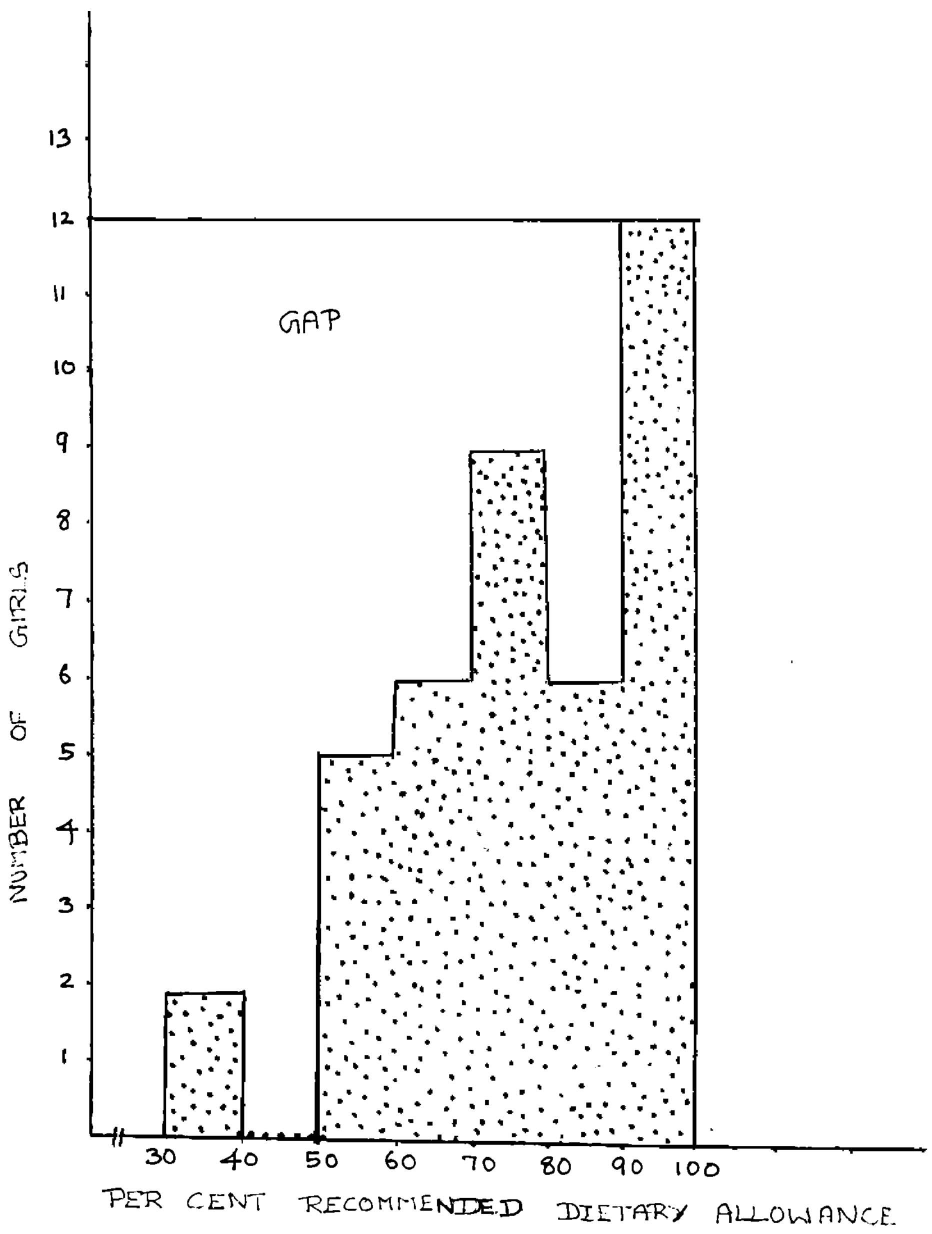
IRON INTAKE OF TEENAGE GIRLS

Number of subjects	Recommended Dietary Allowance ICMR (1982) mg/day	Total iron intake mg/day	Range mg/day
40	35	27.30 ± 3.32	13.42 to 34.16

As depicted in Table-I, the iron intake of the selected teenage girls ranged from 13.42 to 34.16 mg per day with a mean of 28.30 ± 3.32 mg per day which was only 78 per cent of the recommended dietary allowance stipulated by ICMR (1982) for this age group. According to Elsborg (1979) the iron intake of adolescent girls was low. Present investigation also implies that the iron intake of the selected teenage girls was lower than the dietary allowanse recommended by ICMR (1982).

FIGURE-1

TOTAL IRON INTAKE OF TEENAGE GIRLS



A comparison of the iron intake of the teenage girls of the two hostels selected is given in Table-II.

TABLE II

IRON INTAKE OF TEENAGE GIRLS RESIDING IN THE TWO HOSTELS

Groups	Number of Subjects	Recommended Dietary allowance ICMR (1982) mg/day	Total iron intake mg/day	Range mg/day
School girls	20	35	22.96 ± 3.67	13.42 to 28.50
College girls	20	35	31.63 ± 2.96	24.90 to 34.85

The iron intake of the selected teenage girls of the school hostel was 22.96 ± 3.67 mg/day with a range of 13.42 to 28.50 mg/day while those residing in the college hostel was 31.63 ± 2.96 mg/day with a range of 24.90 to 34.85 mg/day. The intake by the school girls was found to be 33.4 per cent below the recommended dietary allowance while it was found to be 9.6 per cent below the recommended dietary allowance stipulated by ICMR (1982) in the case of teenage girls in the college hostel. The iron intake of the selected girls in the college hostel was found to be higher than those residing in the school hostel. This was mainly due to the inclusion of jaggery and a higher amount of green leafy vegetable, amaranth in the menu of the college hostel.

Total, ionizable and soluble iron content of the diets of teenage girls is presented in Table-III.

TABLE - III

TOTAL, SOLUBLE AND IONISABLE IRON OF THE DIETS OF TEENAGE GIRLS

Groups	Total iron mg/100g	Soluble iron+ mg/100g	Ionisable iron+ mg/100g
School girls	7.47 ± 1.16	17.11 ± 2.80	3.87 ± 1.04
College girls	10.96 ± 0.65	22.30 ± 2.43	5.90 ± 0.49

+at p^H 7.5

Total iron determined per 100g of the diet consumed by teenagers of the school hostel and the college hostel was 7.47 ± 1.16 and 10.96 ± 0.65 mg respectively. Total iron content of the college hostel diet was greater than the school hostel diet. The values obtained were found to be similar to the values of Narasinga Rao and Prabhavathi (1978)

Soluble iron determined per 100g of the diet consumed by teenagers of the school and the college hostel was 17.11 ± 2.5 and 22.50 ±2.63 mg respectively. Soluble iron was greater in the college hostel diet than in the school hostel diet. Narasinga Rao and Prabhavathi (1978) obtained a value of 21.8 mg per 100 g of the diet for soluble iron. These values when compared with the values of 17.11 ±2.5 and 22.50 ± 2.63 mg per 100 g of the diet obtained by the present invitro study as soluble iron show a high degree of similarity between the two values.

Ionizable iron determined per 100 g of the diet consumed by teenagers of the school hostel and the college hostel was 3.57 ± 1.04 and 5.90 ± 0.49 mg respectively. Ionizable iron was greater in the college hostel diet than in the school hostel diet. Narasinga Rao and Prabhavathi (1978) also have reported a value of 5.9 mg per 100 g of the diet for ionizable iron. This might be due to the fact that the same invitro technique was employed in both cases for the determination of available iron from foods.

The determination of total iron is only a quantitative approach and that of ionizable iron is only a qualitative one, an effective approach to find out the exact bioavailability of iron from foods is the determination of absolute available iron from them. An attempt was therefore made to determine the absolute available iron which is given in Table-IV.

TABLE-IV

TOTAL, IONIZABLE AND ABSOLUTE AVAILABLE IRON CONTENT OF THE DIETS OF TEENAGE GIRLS

Groups	Total iron mg/100g	Ionizable iron mg/100g	Absolute available iron mg/100g
School girls	7.47 ± 1.16	3.57 ± 1.05	0.279 ± 0.11
College girls	10.96 ± 0.65	5.90 ± 0.49	0.644 ± 0.13

Absolute available iron of the diets of teenage girls of the school and college hostel was 0.279 ± 0.11 and 0.644 ± 0.13 mg per 100g of the diet respectively. The absolute available iron was higher in the college hostel diet than the school hostel diet. This may be due to the fact that the diet intake of the school girls was low and also due to the low iron content of their diet.

The absolute available iron content of the diet of teenage girls of the two hostels selected is presented in Table-V ~~and Figure-2~~ and the individual values are given in Appendix-V.

TABLE-V

ABSOLUTEAVAILABLE IRON CONTENT OF THE DIETS OF TEENAGE GIRLS OF THE TWO HOSTELS

Groups	Number of subjects	Recommended Dietary Allowance ICMR(1982)	Total iron mg/day	Ionizable iron mg/day	Absolute available iron mg/day	Range mg/day
School girls	20	35	22.96 ± 3.67	10.96 ± 3.34	2.82 ± 1.04	0.84 ± to 4.33
College girls	20	30	31.63 ± 2.96	17.51 ± 2.89	4.76 ± 1.74	3.28 to 10.97

The absolute available iron of the selected teenage girls of the school hostel was 2.52 ±1.04 mg per day with a range of 0.84 to 4.33 mg per day while those residing in the college hostel was 4.76 ±1.74 mg per day with a range of 3.33 to 10.97 mg per day. The absolute available iron in the diet of the school girls was founded to be 92.8 per cent below the recommended dietary allowance. While it was found to be 86.4 per cent below the dietary allowance recommended by ICMR (1982) in the case of teenage girls in the college hostel. The absolute available iron of the selected girls in the college hostel was found to be higher than these residing in the school hostel. This was mainly due to the inclusion of jaggery in the college hostel diet which promote the iron absorption. A high deficit in the total iron intake of the selected subjects was noticed. It was 27.30 ±3.32 mg per day which is 78 per cent of the recommended dietary allowance for this age group. But the iron that is available to them is found to be very low. A deficit of 92.8 and 86.4 per cent in the case of school girls and college girls is noticed respectively. This indicated that it is not the iron intake but the availability of iron is the problem in solving the anaemia.

Table-VI gives the total, soluble and ionizable iron content of individual diets consumed by teenage girls and the same is illustrated in Fugure-1.

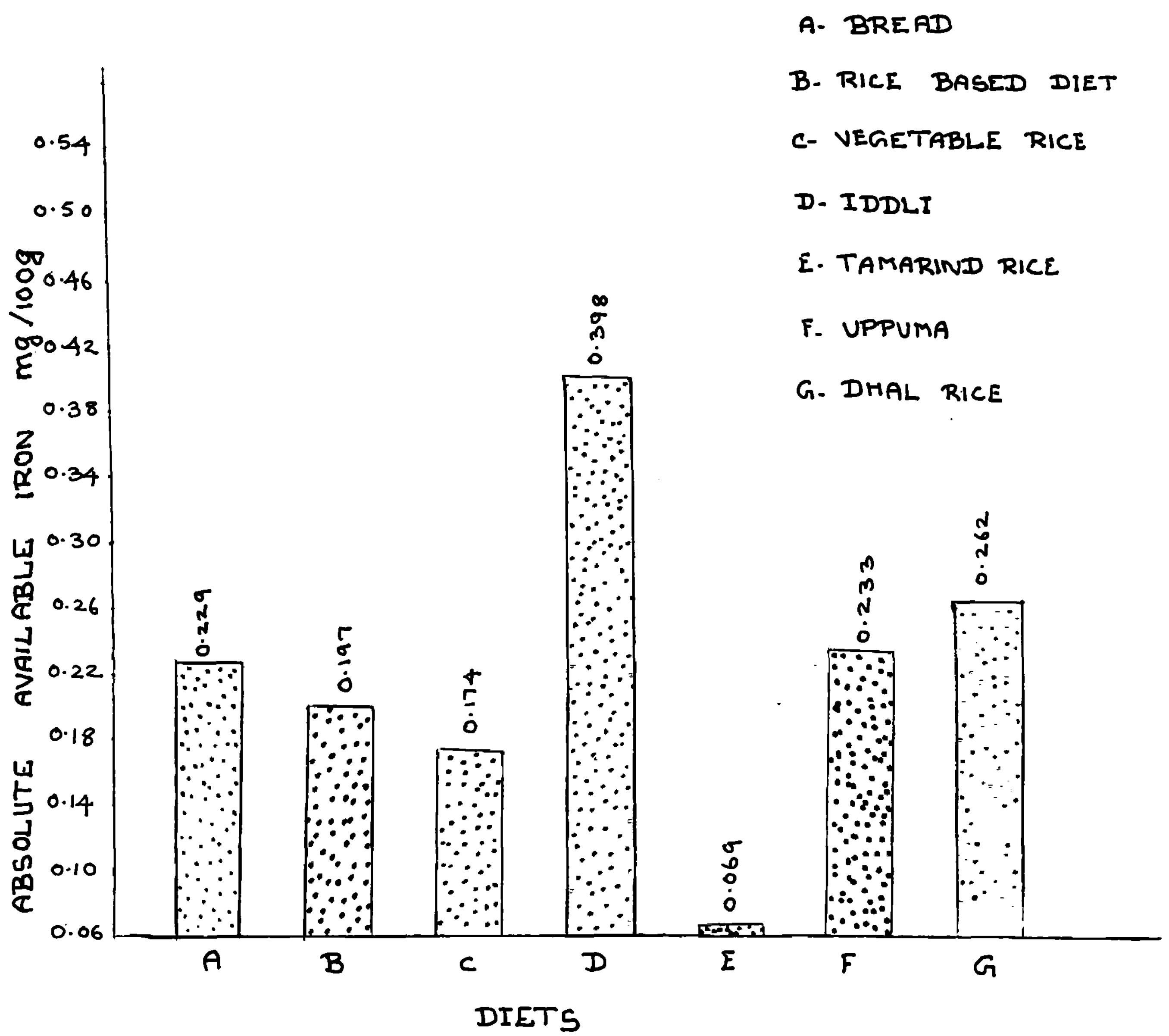
TABLE-VI

TOTAL, SOLUBLE AND IONIZABLE IRON FROM INDIVIDUAL DIETS

Diets	Total iron mg/diet	Soluble iron mg/100g	Ionizable iron mg/100g
Bread	7.32 ± 3.39	18.48 ± 3.60	6.43 ± 1.35
Rice Based diet	9.22 ± 2.57	23.44 ± 2.13	6.89 ± 0.48
Vegetable rice	7.44 ± 3.79	20.16 ± 2.36	7.87 ± 1.22
Iddli	15.81 ± 4.19	19.53 ± 1.15	7.69 ± 0.26
Tamarind rice	5.96 ± 1.60	14.96 ± 2.46	4.70 ± 0.38
Uppuma	7.89 ± 4.22	17.98 ± 1.80	5.75 ± 0.86
Dhal rice	9.41 ± 4.26	23.99 ± 2.00	7.49 ± 0.77

FIGURE-2

ABSOLUTE AVAILABLE IRON CONTENT OF INDIVIDUAL DIETS



Total iron content of bread was 7.32 ± 3.39; rice based diet 9.22 ± 2.37; vegetable rice 7.44 ± 3.79; iddli 13.81 ± 4.19; tamarind rice 5.96 ± 1.60; uppuma 7.89 ± 4.22 and dhal rice 9.41 ± 4.26 mg per diet respectively. Total iron content was greatest in iddli and lowest in tamarind rice. This might be due to the high iron content (9.1 mg per 100g) of black gram dhal used in iddli.

Soluble iron content of bread was 18.40 ± 3.60; rice based diet 23.44 ± 2.13; vegetable rice 25.16 ± 2.36; iddli 19.53 ± 1.15; tamarind rice 14.96 ± 2.48 uppuma 17.98 ± 1.00 and dhal rice 23.99 ± 2.00 mg per 100 g of the diet respectively. Again soluble iron content was found to be greatest in iddli and lowest in tamarind rice.

The ionizable iron content of bread was 6.43 ± 1.58; rice based diet 6.89 ± 0.48 vegetable rice 8.37 ± 1.22; iddli 7.69 ± 0.25 ; tamarind rice 4.70 ± 0.38; uppuma 5.75 ± 0.56 and dhal rice 7.49 ± 0.77 mg per 100 g of the diet respectively. The ionizable iron was highest in iddli and lowest in tamarind rice.

Eventhough tamarind contains 10.9 mg of iron per 100g the total, soluble and ionizable iron content of the tamarind rice was low. This may be due to the presenck of tannin which forms insoluble iron-tannate complexes and makes the iron unavailable to the system.

Table-VII gives the total, ionizable and absolute available iron content of the individual diets consumed by teenage girls.

TABLE-VII

TOTAL, IONIZABLE AND ABSOLUTE AVAILABLE IRON CONTENT OF THE INDIVIDUAL DIETS

Diets	Total iron mg/100g	Ionizable iron mg/100g	Absolute available iron mg/100g
Bread	3.48 ± 0.36	6.43 ± 1.39	0.229 ± 0.130
Rice based diet	2.47 ± 0.46	6.89 ± 0.48	0.197 ± 0.069
Vegetable rice	1.84 ± 0.43	7.57 ± 1.32	0.174 ± 0.059
Iddli	3.12 ± 0.62	7.69 ± 0.25	0.398 ± 0.069
Tamarind rice	1.45 ± 0.31	4.70 ± 0.38	0.069 ± 0.086
Uppuma	4.06 ± 0.71	5.75 ± 0.56	0.233 ± 0.057
Dhal rice	2.61 ± 0.24	7.49 ± 0.77	0.262 ± 0.053

The absolute available iron of individual diets such as bread, rice based diet, vegetable rice, iddli, tamarind rice, uppuma and dhal rice was 0.229 ± 0.130; 0.197 ± 0.039; 0.174 ± 0.039; 0.398 ± 0.059; 0.069 ± 0.055; 0.233 ± 0.057 and 0.262 ± 0.063 mg per 100 g of the diet respectively. The absolute available iron content was highest in iddli and lowest in tamarind rice. Iron content of black gram dhal which is used in iddli and tamarind was 9.1 and 10.9 mg per 100g respectively. Eventhough the iron content is high in tamarind due to the small amounts of tamarind used and the presence of tannin in tamarind which forms the insoluble iron-tannate complexes, the absolute available iron content is lowest in tamarind rice. Due to fermentation effect in iddli the pH will be lowered. At acidic pH solubility of iron increases considerable and the proportion of ionizable iron is very high (Narasinga Rao and Prabhavathi, 1978). This might be the reason for the high content of absolute iron in iddli.

TABLE - VIII

EFFECT OF LIME JUICE ON IONIZABLE IRON

Diets	Amount of ascorbic acid added mg	Ionizable iron before the addition of ascorbic acid mg/100g	Ionizable iron after the addition of ascorbic acid mg/100g	Increment in iron content mg/100g	Per cent increase %	't' value
Iddli	5.52	7.69 ± 0.25	12.66 ± 1.56	4.18 ± 1.76	64.65	3.152**
Bread	5.52	6.43 ± 1.55	9.71 ± 0.57	4.97 ± 1.59	51.01	6.272**
Uppuma	5.52	5.75 ± 0.56	7.99 ± 0.82	2.31 ± 1.00	39.86	10.06**

** ($P < 0.01$)

Table-VIII gives the effect of lime juice on ionizable iron at p^H 7.5.

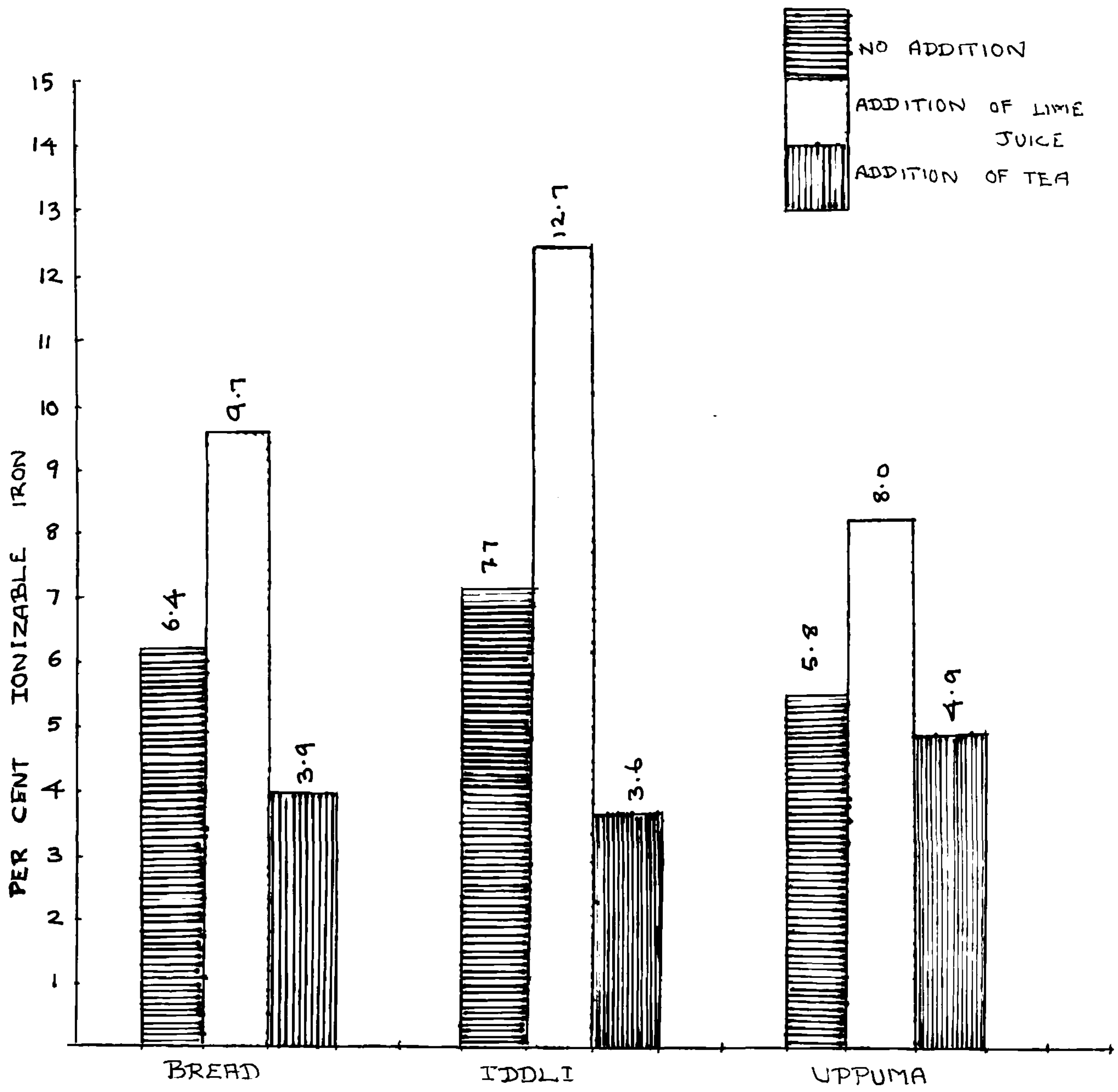
Since ascorbic acid content of the diets such as iddli, bread, and uppuma was nil these breakfast items were selected to study the effect of lime juice (ascorbic acid) on ionizable iron. The ionizable iron content of the diets such as iddli, bread and uppuma was estimated before and after the addition of lime juice to the diets. In all the three cases the ionizable iron content was increased significanly ($P < 0.01$). Addition of lime juice containing 5.52 mg of ascorbic acid to iddli increased the ionizable iron from 7.69 ± 0.25 to 12.66 ± 1.56 mg per 100g of the diet which shows an increment of 4.18 ± 1.76 mg of ionizable iron per 100g of the diet that is there is 64.65 per cent increase., An ionizable iron content of iddli after the addition of ascorbic acid to it. In bread the ionizable iron content was increased from 6.43 ± 1.55 to 9.71 ± 0.57 mg per 100g of the diet which shows an increment of 4.97 ± 1.59 mg of ionizable iron per 100g of the diet, that is, there is an increase in ionizable iron content by about 51.01 per cent. In the case of uppuma the ionizable iron increased from

5.75 ± 0.36 to 7.99 ± 0.82 mg per 100g of the diet which shows an increment of 2.31 ± 1.00 mg of ionizable iron per 100g of the diet, that is, the ionizable iron content is increased by about 39.86 per cent. Rathee and Pradhan (1979) also found that ascorbic acid markedlyincreasedfood iron absorption from 18.90 ± 1.05 to 26.40 ± 2.05 mg per 100g of the diet. This effect is due to a promotion of nonheme iron absorption and there is no effect on the absorption of heme iron. The increase in absorption is related to the amount of ascorbic acid. Hence it is advisable to take lime juice along with breakfast. Figure-3 shows the effect of lime juice and tea on ionizable iron of the selected breakfast items.

Table-IX presents the effect of tea on ionizable iron. Since tannin content of uppuma bread and iddli was nil these breakfast items were selected to study the effect of tea on ionizable iron at p^H 7.5

FIGURE-3

EFFECT OF LIME JUICE AND TEA ON IONIZABLE IRON



TABLE–IX

EFFECT OF TEA ON IONIZABLE IRON

Diets	Amount of tannin added mg	Ionizable iron before the addition of tannin mg/100g	Ionizable iron after the addition of tannin mg/100g	Decrease in iron content mg/100g	Percent decrease %	't' value
Uppuma	577.5	5.75 ± 0.56	4.88 ± 0.63	2.53 ± 1.28	15.13	10.17**
Bread	577.5	6.43 ± 1.55	3.90 ± 0.54	4.07 ± 0.32	39.34	4.87**
Iddli	577.5	7.69 ± 0.25	3.63 ± 0.39	4.99 ± 0.69	52.79	27.73**

** ($P < 0.01$)

The ionizable iron content of the diets such as uppuma, bread and iddli was estimated before and after the addition of tea to the diet. Addition of the tea containing 377.5 mg of tannin to uppuma decreases the ionizable iron from 5.73 ± 0.56 to 4.88 ± 0.63 mg per 100g of the diet which equals a decrease of 2.33 ± 1.28 mg of ionizable iron per 100g of the diet, that is, the decrease was 15.13 per cent in the case of bread the decrease was from 6.43 ± 1.55 to 3.90 ± 0.54 mg per 100g of the diet which equals a decrease of 4.07 ± 0.32 mg of ionizable iron per 100g of the diet that is, the decrease was 39.34 per cent. The ionizable iron decreased from 7.69 ± 0.25 to 3.63 ± 0.39 mg per 100g of iddli whichequals a decrease of 4.99 ± 0.64 mg that is, the decrease was about 52.79 per cent. These results correlate well with the results of NIN (1979) which shows that tea containing 300g of tannin markedly reduced the absorption of non-heme iron from 6.35 to 2.39 mg per 100g of bengal gram seeds. This effect has been ascribed to the formation of iron tannate complexes. In all the three cases the decrease was found to be significant ($P < 0.01$). Hence it is advisable to avoid tea along with breakfast.

V. SUMMARY AND CONCLUSION

The bioavailability of iron from diets consumed by 40 teenage girls selected at random from two hos-tels was investigated using an *invitro* method. The diets consumed by the above nutritionally vulnerable groups were collected for a period of three days and 10 per cent of the food consumed by a single individual was taken as a sample for analysis.

The invitro bioavailability of iron from individual meals and the effect of tea and lime juice on iron absorption in breakfast meals have also been studied.

The conclusions arising out of this study are:

1. The iron intake of the selected teenage girls ranged from 13.42 to 34.16 mg per day with a mean of 27.30 ± 3.32 mg per day which was only 78 per cent of the recommended dietary allowance stipulated by ICMR (1982) for this age group.

2. The iron intake of the selected teenage girls of the school hostel was 22.96 ± 3.67 mg per day with a range of 13.42 to 28.50 mg per day, while those residing in the college hostel was 31.83 ± 2.96 mg per day with a range of 24.90 to 34.85 per day. The

intake by the school girls was found to be 23.4 per cent below the recommended dietary allowance while it was 9.6 per cent below the recommended dietary allowance stipulated by ICMR (1982) in the case of teenage girls in the college hostel. The iron intake of the selected girls in the college hostel was found to be higher than those residing in the school hostel. This was mainly die to the inclusion of jaggary and a higher amount of green leafy vegetable, amaranth in the menu of the college hostel.

3. Total iron determined per 100g of the diet consumed by the teenagers of the school hostel and the college hostel was 7.47 ±1.16 and 10.96 ± 0.65 mg respectively. Total iron content of the college hostel diet was greater than the school hostel diet.

Soluble iron determined per 100g of the diet condumed by the teenagers of the school hostel and the college hostel was 17.11 ±2.00 and 22.30 ±2.63 mg respectively. Soluble iron was greater in the college hostel diet than in the school hostel diet.

Ionizable iron determined per 100 g of the diet consumed by the teenagers of the school hostel and the college hostel was 3.57 ± 1.04 and 5.90 ± 0.49 mg respectively. Ionizable iron was greater in the college hostel diet than in the school hostel diet.

4. Absolute available iron of the diets of the teenage girls of the school and college hostel was 0.279 ± 0.11 and 0.644 ± 0.13 mg per 100 g of the diet respectively. The absolute available iron was higher in the college hostel diet than the school hostel diet. This may be due to the fact that the diet intake of the school girls was low and also due to the low iron content of their diet.

5. The absolute available iron of the selected teenage girls of the school hostel was 2.52 ± 1.04 mg per day with a range of 0.84 to 4.33 mg while those residing in the college hostel was 4.76 ± 1.74 mg per day with a range of 3.33 to 10.97 mg per day.

6. Total iron content of bread was 7.32 ± 3.39 rice based diet 9.22 ± 2.57; vegetable rice 7.44 ± 3.79; iddli 15.81 ± 4.19; tamarind rice 5.96 ± 1.60; uppuma 7.89 ± 4.32 and dhal rice 9.41 ± 4.26 mg per diet respectively. Total iron content was greatest

in iddili and lowest in tamarind rice. This might be due to the high iron content (9.1 mg per 100g) of black gram dhal used in iddli.

Soluble iron content of bread was 18.4± 3.6; rice based diet 23.44± 2.13; vegetable rice 25.16± 2.56; iddli 19.53± 1.15; tamarind rice 14.96± 2.45; uppuma 17.98± 1.80 and dhal rice 23.99± 2.00 mg per 100g of the diet respectively. Again soluble iron content was found to be greatest iniddli and lowest in tamarind rice.

The ionizable iron content of bread was 6.43± 1.55; rice based diet 6.89± 0.48; vegetable rice 8.57± 1.32; iddli 7.69± 0.25; tamarind rice 4.70± 0.38; uppuma 5.75± 0.56 and dhal rice 7.49± 0.77 mg per 100g of the diet respectively. The ionizable iron content is lowest in tamarind rice and highest in iddli. Eventhough tamarind contains 10.9 mg of iron per 100g, the total, soluble and ionizable iron content of the tamarind rice was low. This may be due to the presence of tannin which forms insoluble iron-tannic which forms insoluble iron-tannate complexes and makes the iron unavailable to the system.

7. The absolute available iron of individual diets such as bread, rice based diet, vegetable rice, iddli tamarind rice, uppuma and dhal rice was 0.229 $\pm$ 0.13 0.197 $\pm$ 0.039; 0.174 $\pm$ 0.059; 0.298 $\pm$ 0.059; 0.069 $\pm$ 0.056 0.233 $\pm$ 0.057 and 0.262 $\pm$ 0.053 mg per 100g of the diet respectively. The absolute available iron content was highest in iddli and lowest in tamarind rice. Iron content of black gram dhal which is used in iddli tamarind was 9.1 and 10.9 mg per 100g respectively. Eventhough the iron content is high in tamarind due to the small amounts of tamarind used and the presence of tannin in tamarind which forms the insoluble iron-tannate complexes, the absolute available iron content is lowest in tamarind rice. Due to fermentation effect in iddli the p^H will be lowered. At acidic pH solubility of iron increases considerably and the proportion of ionizable iron is high. This might be the reason for the high content of absolute available iron in iddli.

8. The ionizable iron content of the diets such as iddli, bread and uppuma was estimated before and after the addition of lime juice to the diets. In all the three cases the ionizable iron content was

increased significantly ($p < 0.01$). Addition of lime juice containing 5.52 mg of ascorbic acid to iddli increased the ionizable iron from 7.69 ± 0.25 to 12.66 ± 1.56 mg per 100 g of iddli which shows an increment of 4.18 ± 1.76 mg of ionizable iron per 100g of iddli, that is a 64.65 per cent increase in ionizable iron content of iddli was noticed after the addition of ascorbic acid to it. In bread the ionizable iron content was increased from 6.43 ± 1.55 to 9.71 ± 0.57 mg per 100g of the diet which shows an increment of 4.07 ± 1.59 mg of ionizable iron per 100g of the diet, that is, there is an increase in ionizable iron content by about 51.01 per cent. In the case of uppuma the ionizable iron increased from 5.75 ± 0.56 to 7.99 ± 0.82 mg per 100g of the diet which shows an increment of 2.37 ± 1.00 g of ionizable iron per 100 g of the diet, that is, the ionizable iron content is increased by about 39.86 per cent. This effect might be due to a promotion of non-heme iron absorption. Hence it is advisable to take lime juice along with breakfast.

9. The ionizable iron content of the diets such as uppuma, bread and iddli was estimated before and after the addition of tea to the diet. Addition of tea containing 577-5 mg of tannin to uppuma decreased the ionizable iron from 5.75± 0.56 to 4.88± 0.63 mg per 100g of the diet which equals a decrease of 2.53± 1.28 mg of ionizable iron per 100g of the diet, thatis, the decrease was 15.13 per cent. In the case of bread the decrease is from 6.43± 1.55 to 3.90± 0.54 mg per 100g of the diet which equals a decrease of 4.07± 0.32 mg of ionizable iron per 100g of the diet, that is, the decrease was 39.34 per cent. The ionizable iron decreased from 7.69± 0.25 to 3.63± 0.39 mg per 100 g of iddli which equals a decrease of 4.99 ± 0.64 mg that is, the decrease was about 52.79 per cent. This might be due to the formaion of iron-tannate complexes, In all the three cases the decrease was found to be significant ($P<0.01$). Hence it is advisable to avoid tea along with breakfast.

APPENDICES

APPENDIX-I

Invitro Iron Availability

(α,α-Dipyridyl Method)

Ionisable iron and soluble iron content of the foods were determined by the invitro technique of Narasinga Rao and Prabhavathi (1978).

Principle

The method is based on stimulation of conditions that exist in the human digestive tract. The method consists of extracting the homogenised food with pepsin hydrochloric acid at P^H 1.35 followed by adjusting the P^H to 7.5. This approximates the conditions in the stomach followed by conditions in small intestine. Ferrous iron reacts with α,α-dipyridyl to give a pink colour which is estimated colorimetrically. Ionisable iron at P^H 7.5 was taken as a measure of iron availability. The final values are expressed as ionisable iron per 100 units of total iron in a food (Absolute available iron)

Reagents

1. α,α-dipyridyl 0.1 per cent in 3 per cent acetic acid
2. 0.1M Sodium sulphate solution (1.26g of anhydrous sodium sulphate in water and make upto 100 ml with water) prepare freshly every few days.
3. Chloroform

4. Standard iron solution (0.7022g of ferrous ammonium sulphate in one litre of deionised water (1ml = 0.1mg of iron)

5. Working standard prepared by diluting 5.0ml of the stock solution to 100ml with water to obtain a solution containing 5 µg per ml.

<u>Procedure</u>

Pipetted out 0.4 to 2.0 ml of the standard iron solutions into clean dry tubes (corresponding to 100-500 ug per 100ml) and made up the volume to 2.0ml with deionised water. 2.0ml of the experimental solution was pipetted out into clean dry tubes. To all the tubes added 2.0ml each of 0.1M sodium sulphate and 2.0ml of α,α- dipyridyl reagent. Heated in a boiling water bath for 5 minutes, cooled in ice for about 5 minutes and added 1.0ml of cholorofora and shook vigorously for 30 seconds. Centrifuged at 3000 r.p.m. for 5 minutes. The colour developed was read in a colorimeter at 520 nm against a reagent blank. The difference between the test solutions and sample blank gives the amount of ionisable iron present in the test solution. The ionisable iron graph was drawn by plottiong Klett readings on Y-axis and concentration of iron on X-axis

APPENDIX-II

Estimation of Iron (NIN 1971)

Thiocyanate method (Wong's Method.)

Principle:

Ferric iron when treated with thiocyanate will give a blood red colour which can be read colorimetrically using 540 mμ filter.

Reagents:

1. 30 per cent sulphuric acid A.R (30.0 ml of concentrated sulphuric acid diluted to 100 ml with water).

2. Saturated potassium persulphate solution: 7.0 g of potassium presulphate (A.R) was dissolved in deionized water and the solution was made upto 100 ml.

3. Potassium Thiocyanate 40 per cent solution: 40.0 g of potassium thiocyanate was dissoved in 50 ml of glass distilled water: 4.0 ml of acetone was added and the volume was made upto 100 ml.

4. Standard iron solution:

0.7022g (A.R) Ferrous ammonium sulphate was dissoved in 100 ml of deionized water and after addition of 5.0 ml of 1:1 hydrochloric acid, the solution was made upto one litre and mixed throughly (1.0 ml = 0.1 mg iron). The standard solution was prepared once in six months.

working standard solution: (0.01 mg iron per ml) was prepared by diluting the above solution ten fold.

Procedure:

1.0, 2.0, 3.0, 4.0, and 5.0 ml of the working standard iron solution was pipetted out into a series of test tubes and 2.0 ml of experimental solutions. To all the tubes added 0.3 ml of concentrated sulphuric acid, 0.4 ml of saturated potassium persulphate and 1.6 ml of potassiumthiocyanate and made up the volume to 100 ml with distilled water. The red colour develped was read in a colorimeter against a reagent blank using a 540 mμ filter within ten minutes. A standard graph was drawn by plotting the concentration of iron on the x-axis and colorimeter reading on the Y - axis.

APPENDIX-III

Estimation of Ascorbic Acid

Dye Method

Principle:

This method depends on the stoichiometric reduction of the dye 2,6 dichlorophenol indophenol to its colourless leuco form. The titration is carried out in presence of oxalic acid in order to inhibit aerobic oxidation catalysed by certain metal ions.

This simple titrimetric method is applicable to the determination of ascorbic acid only in the absence of other reducing substances and where only the reduced form of the vitamin is present. Thus it may be applied to fresh orange, grape fruit, lime or tomato juice and some pharmaceutical preparations.

Reagents:

1. 2,6 dichlophenol indophenol solution - Dissolved 42.0mg of sodium bicarbonate and 52.0 mg of the dye in 50 ml of water. Diluted to 200 ml. Filtered and stored in the refrigerator for not more than 3 days.

2. Standard ascorbic acid solution:-

100 mg of pure dry ascorbic acid crystals were

dissolved and made upto 100 ml in a standard flask with 4% oxalic acid solution. 10.0 ml of it was diluted to 100 ml with 4% oxalic acid solution so that 1.0 ml of it contains 0-1 mg of ascorbic acid.

Procedure:

Standardisation of the dye:

10.0 ml of the standard ascorbic acid solution was pipetted out into a clean dry conical flask and titrated against the dye taken in the burette. The end point was the appearance of a pale pink colour that persisted for 5 seconds. From the amount of the dye consumed, calculated and expressed the strength of the ~~dye-so~~ indophenol solution as mg ascorbic acid equivalent per ml reagent.

The fresh lime was cutand squeezed out the juice. 5.0 ml of it was made upto 100 ml in a standard flask with 4% oxalic acid. Pipetted out 5.0 ml of it into a clean conicial flask and titrated it against the dye as above.

APPENDIX - IV

Estimation of Tannins (AOAC - 1965).

Principle:

The method is based on the fact that phosphotungstomolybdic acid is reduced by tannin like compounds in alkaline solution producing a highly coloured blue solution, the intensity of which is measured spectrophotometrically using a red filter.

Reagents:

1. Folin - Denis Reagent:-

To 100 g solution sodium tungstate, 20 g phosphomolybdic acid , 50 ml phosphoric acid, and 750 ml distilled water were added. The mixture was allowed to reflux for two hours and made upto one litre.

2. Sodium carbonate solution:

350 g sodium carbonate was dissolved in one litre water at 70-80 c. Filtered through glass wool after allowing it to stand overnight.

3. Standard tannic acid solution: (0.1%)

100 mg of the stock tannic acid solution. was dissolved in 100 ml of distilled water.

4. Working standard tannic acid solution.

5.0ml of the stock tannic acid solution was diluted to 100 ml with distilled water 1 ml = 50 μg of tannin

Procedure:

1. Weighed 1g of the powdered material four times and transferred to 500 ml conical flask. Added 150 ml water to each flask.
2. Then added to three Flasks respectively 10, 15, and 20 ml of standard tannic acid solution.
3. Heated all the Flasks gently and boiled for 30 minutes Then centrifuged at 2000 r.p.m. for 20 minutes.
4. Collected supernatant in 250 ml flasks and made up the volume.
5. Transferred 10ml of the supernatant extract in 100ml flasks and added 75 ml do of water, 8.5 ml of Folin-Denis reagent and 5.0ml of standard sodium carbonate solution and made up the volume.
6. After 30 minutes took the reading at 740 nm.
7. Prepared a graph by plotting O.D. versus tannic acid concentration. The value wherever it cuts the X-axis was taken as new origin. The difference in O.D. in first and second origin was taken as a measure of the content of tannins in the samples.

APPENDIX -V

Total, ionizable and absolute available iron content of the diets of teenage girls.

Serial Number	Total iron mg/day	Ionizable iron mg/day	Absolute available iron mg/day
1	22.08	5.75	1.27
2	18.06	9.80	1.77
3	19.86	8.20	1.63
4	20.58	8.82	1.82
5	26.08	8.44	2.20
6	18.80	4.47	0.84
7	20.26	8.43	1.71
8	23.57	12.37	2.94
9	24.66	10.84	2.67
10	24.95	11.38	2.84
11	27.13	11.40	3.09
12	25.31	10.51	2.66
13	26.12	11.59	3.03
14	28.50	15.18	4.33
15	23.07	11.10	2.56
16	22.33	10.43	2.33
17	27.41	15.61	4.26
18	23.40	13.21	3.09
19	13.42	7.63	1.02
20	23.65	10.51	4.41
21	31.73	12.82	4.31
22	32.78	9.83	5.88
23	30.97	10.29	5.28
24	31.71	11.01	5.84

Serial Number	Total iron mg/day	Ionisable iron mg/day	Absolute available iron mg/day
25	32.47	9.73	6.35
26	33.28	11.78	5.68
27	32.43	11.23	5.81
28	32.33	9.70	6.77
29	33.83	11.65	5.22
30	28.70	8.52	5.53
31	27.19	14.16	3.33
32	24.90	7.47	5.80
33	28.13	8.44	5.78
34	30.01	9.00	5.79
35	34.06	10.52	5.23
36	34.16	13.23	10.97
37	30.51	12.15	4.13
38	33.84	10.75	6.65
39	34.80	13.43	5.34
40	34.85	13.46	4.96

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