STUDIES ON THE BIOAVAILABILITY OF MICRONUTRIENTS FROM INDIAN FOODS

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UNIVERSITY OF MYSORE

For the award of the degree of
DOCTOR OF PHILOSOPHY
in
Food Science and Nutrition

by
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DECLARATION

I hereby declare that this thesis entitled "**STUDIES ON THE BIOAVAILABILITY OF MICRONUTRIENTS FROM INDIAN FOODS"** submitted to the **UNIVERSITY OF MYSORE**, for the award of the degree of **DOCTOR OF PHILOSOPHY** in **FOOD SCIENCE AND NUTRITION**, is the result of research work carried out by me in the Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, under the guidance of **Dr. K.Srinivasan** during the period May 2002-January 2006. I further declare that the work embodied in this thesis is original and has not been submitted previously for the award of any degree, diploma or any other similar title.

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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON THE BIOAVAILABILITY OF MICRONUTRIENTS FROM INDIAN FOODS."** submitted by **Mrs. Hemalatha S.** to the University of Mysore for the award of the degree of **'Doctor of Philosophy' in Food Science and Nutrition**, is the result of work carried out by her in the Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, under my guidance during the period May 2002 - January 2006.

K.SRINIVASAN
(Guide)

Place: Mysore

Date:

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LIST OF ABBREVIATIONS

Conc.	Concentration
L	Litre
ml	Milliliter
g	Gram
mg	Milligram
h	Hour
min	Minute
kDa	Kilodaltons
^{o}C	Degree centigrade
Psi	Pounds per square inch
U	Units
M	Molar
HCl	Hydrochloric acid
$CaCO_3$	Calcium carbonate
BOD incubator	Biological Oxygen Demand incubator
RDA	Recommended Dietary Allowance
WHO	World Health Organization
ICMR	Indian Council of Medical Research
%	Per cent
vs.	Versus
viz.	Namely
Fig.	Figure
i.e.	That is

CHAPTER – I

GENERAL INTRODUCTION

General Introduction

Micronutrients

Nutrients that are generally present at relatively low concentrations in living tissues and which are needed for optimum performance of a particular function in the living system are designated as ‘Micronutrients’. They include vitamins and minerals. Micronutrients, although do not provide energy themselves unlike macronutrients (carbohydrate, fat and protein), help in the liberation and utilization of energy from the macronutrients. In addition, they have other specific vital functions in the body. Vitamins are essential for the utilization of the macronutrients and also in the regulation of the body processes – metabolism and circulation, digestion and absorption of nutrients and reproduction. Macrominerals such as calcium and phosphorous are structural components of bones and skeleton; sodium and potassium are elements present in cellular fluids; magnesium is an active constituent of many enzyme systems. Among the microminerals, iron is an essential component of red blood cells; zinc, molybdenum, copper and manganese activate a number of enzyme systems essential for diverse body functions; iodine is a part of the hormone thyroxine. Other trace elements either essential or beneficial to mammalian and avian species include: arsenic, boron, chromium, cobalt, fluorine, nickel, selenium, silicon and vanadium (Berdaneir, 1997)

Trace elements exist in two forms- as charged ions or bound to proteins (e.g., Metalloenzymes). Each trace mineral has a different chemical property and hence each element is required for a particular function in the living system (Anderson, 2000). Among the micro minerals, iron has drawn the attention of many researchers in view of the widespread deficiency of this mineral among the population of the world. Zinc is gaining increased attention in recent years, because the deficiency of this element is believed to be equally widespread, with consequences as severe as those of iron deficiency.

Iron

Distribution of iron in the body:

Iron in human body is present as two major pools- functional and storage iron. Functional iron - in hemoglobin (60%), myoglobin (5%) and in various heme (cytochromes and catalase) and non-heme (NADH hydrogenase, succinic dehydrogenase, aconitase) enzymes (5 %); Storage iron - in ferritin (20%), hemosiderin (10%) and transferrin (0.1%), the major iron transport protein in blood.

The total iron content of an adult man is about 3.6 g, while that of a woman is 2.4 g (Table-1). Women have lesser stores of iron than do men. Iron is highly conserved in the body, 90% of it being recovered and reused every day, while only 10% of it is excreted, mainly in bile (Anderson, 2000).

Absorption:

The body has a unique mechanism for maintaining iron balance that prevents iron deficiency and overload. The mechanism involves three steps namely, storage, reutilization and regulation of iron absorption. Dietary iron is present in two forms - as heme iron in animal foods and non-heme iron in plant foods and as non-heme enzymes and ferritin (in some animal foods).

Non-heme iron from food is absorbed mainly in the duodenum by an active process that transports iron from the gut lumen into the mucosal cell (Fig.1). When the body requires iron, it passes directly through the mucosal cell into the blood stream, where it is transported by transferrin, together with the iron released from old blood cells. If iron is not required by the body for metabolic processes, the absorbed iron is stored in the mucosal cell as ferritin and is excreted in feces when the mucosal cell is exfoliated. Excess absorbed iron is also stored as ferritin or hemosiderin in liver, spleen or bone marrow. The absorption of non-heme iron is strongly influenced by dietary components which bind iron in the intestinal lumen rendering it unavailable for absorption if the complex is insoluble; alternatively, facilitating iron absorption if the complex is soluble.

Table-1 Relative proportions of iron in adult humans

	Men		Women	
	mg	**%**	**mg**	**%**
Functional				
Hemoglobin	2300	64	1700	73
Myoglobin	320	9	180	8
Heme enzymes	80	2	60	3
Non-heme enzymes	100	3	80	3
Storage				
Ferritin	540	15	250	9
Hemosiderin	230	6	100	4
Transferrin	5	<1	4	<1
Total	**2375**		**2314**	

Source: Anderson, 2000

Heme iron is absorbed by a different mechanism. The heme iron is absorbed intact into the mucosal cell as iron-porphyrin complex, where the enzyme heme oxygenase releases iron from this complex (Fig.1). Its absorption is little influenced by dietary components, whereas the iron status of the individual has a bigger influence on the same (Hallberg, 1981). The absorption of heme iron into the mucosal cells is independent of non-heme iron. Non-heme iron and heme iron then seem to have a common pathway out of the mucosal cells into the plasma. This difference in the absorption mechanism of the two kinds of iron may be the probable reason why heme iron is much less influenced by the iron status of the subject (Hallberg, 1981).

Although heme iron represents only 10-15% of the dietary iron in populations with a high meat intake, it could contribute as much as 40% of the iron absorbed, since its absorption is almost complete. The absorption of non-heme iron, on the other hand, is only about 1 to 20%, depending on the relative proportion of inhibitors and enhancers of iron absorption in the diet, thus necessitating a higher consumption of iron by populations dependent on plant foods. Factors influencing the absorption of heme and non-heme iron are summarized in Table-2.

Functions of iron:

Iron is required for a broad spectrum of metabolic functions. As a part of hemoglobin, iron is required as transporter of oxygen for respiration and as a part of myoglobin it is needed for oxygen storage in muscle. This element is required in red blood cell function, myoglobin activity, and is a part of many heme and non-heme enzymes.

Oxidative production of ATP within the mitochondria involves many iron-containing enzymes. The cytochromes present in cell function in the respiratory chain, participating in the transfer of electrons and storage of energy through alternate oxidation and reduction (Anderson, 2000).

$$Fe^{++} \longleftrightarrow Fe^{+++}$$

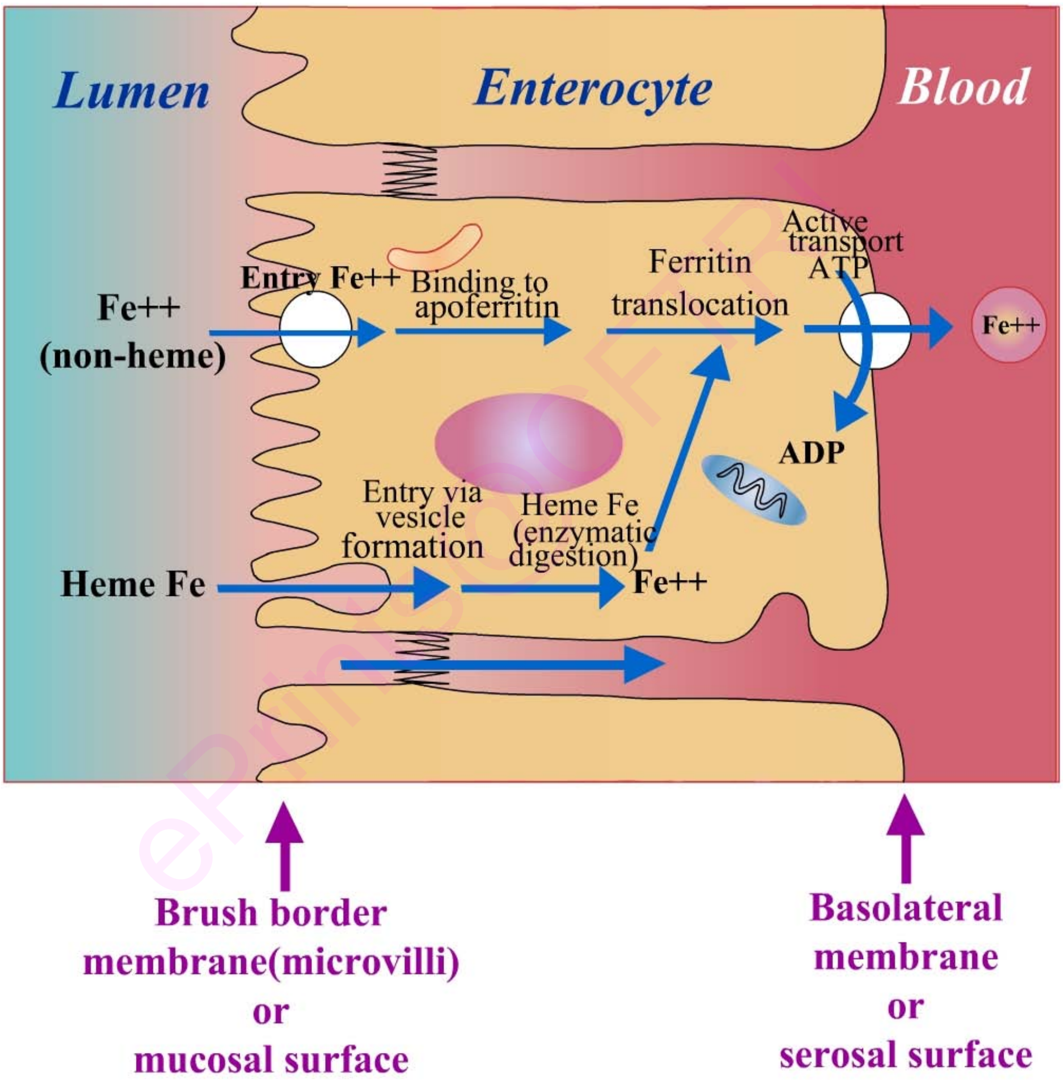


Fig. 1 Absorption of heme and non-heme iron by the intestinal mucosal cell.

Source: Anderson, 2000

Table-2 Factors influencing iron absorption

Increased absorption	Decreased absorption
(a)Heme	
Physiological factors	
Low iron status	High iron status
Degree of gastric secretions	Increased intestinal motility
Dietary factors	
Low heme iron intake	High heme iron intake
Meat	Calcium
(b) Non- heme iron	
Physiological factors	
Depleted iron status	Repleted iron states
Pregnancy	Achlorohydria
Growth	
Depleted iron stores	
Disease states such as Anemias	
Dietary factors	
Ascorbic acid	Phytate
Animal foods	Tannins
Organic acids	Calcium
Lacto ferritin	
Lactalbumin	
Ferrous form of salts (supplements)	
Particle size of iron	
Tannin	

Source: Strain and Cashman, 2003

Iron is used by the brain cells for normal functions (Beard *et al*, 1993). It is involved in cognitive performance; several studies have reported poor scholastic performance, sensory-motor competence, attention, learning and memory of anemic children (Scrimshaw, 1994). Iron is involved in the function and synthesis of neurotransmitters and also myelin. Two iron binding proteins, transferrin and lactoferrin protect against infection by making the element unavailable for microorganisms. Iron is also involved in immune function and reproductive performance.

Dietary sources:

Beef, poultry, clams, oysters, fish, lamb, liver, and pork are good dietary sources of heme iron. The dietary sources of non-heme iron include eggs, dried legumes, green leafy vegetables and cereals. The RDA for iron as suggested by the ICMR varies between 12-30 mg/day for different age groups, except in pregnancy when it is 38 mg/day.

Deficiency:

Iron deficiency results in a host of abnormalities, the most severe condition being iron-deficiency anaemia. Iron- deficiency anaemia is a major public health problem and is prevalent globally. It is estimated that nearly 66-80% of the world's population is iron deficient, of which around 30% is anaemic (WHO, 2002). In South East Asia the number affected by iron deficiency is highest, which includes 24.8 million pregnant women and 111.4 million children under the age of 5 years.

While mild to moderate deficiency of iron causes non-specific symptoms such as fatigue, pallor, headache, dyspnea and palpitations, iron- deficiency anaemia results in serious consequences such as subdued immuno - competence, reduced cognitive performance, poor reproductive performance and lowered work capacity (Anderson, 2000).

Iron deficiency results from either low intake of dietary iron or due to its poor absorbability from plant foods. Intestinal parasites like hookworm, whipworm and roundworm also cause anaemia. Schistoma infection, malaria, thalassemia and chronic infections like tuberculosis and AIDS are some of the other causes of anemia (Florentino, 2003).

Iron toxicity:

Overload of body tissues with iron is usually rare because of the very effective regulation of iron absorption. However, overuse of iron supplements could pose a possible risk of iron toxicity. The symptoms of such a situation include intestinal damage with haemorrhagic episodes in the gastrointestinal tract, vomiting and some times hepatic insufficiency. If the iron levels exceed 200-250 mg/kg body weight, it is fatal. Abnormal accumulation of iron in hair and liver, excessive tissue ferritin levels, oxidation of low-density lipoprotein cholesterol and cardiovascular complications are some of the clinical manifestations of iron overload. A condition called hemosiderosis develops when ingestion of iron is excessive and abnormally accumulated or from a genetic disorder, hemochromatosis. Frequent blood transfusions may also lead to overload of iron (Berdaneir, 1997). In addition to these, excess iron may result in generation of free radicals and thus increase the carcinogenic molecules in the cells (Anderson, 2000).

Zinc

Zinc is one of the most important trace elements in the body. It is found in all body tissues and fluids in relatively high concentrations, with 85% of the whole body zinc in muscle and bone, 11% in the skin and liver and the remaining in the other tissues. The average amount of zinc in the adult body is about 1.4 - 2.3 g (Calesnick & Dinan, 1988). This mineral is indispensable for the growth and development of not only animals, but also microorganisms and plants. In multicellular organisms, zinc is entirely intracellular, 40% being located in the nucleus, 50% in the cytoplasm, organelles and specialized vesicles and the remaining in the cell membrane (Tapiero & Tew, 2003).

Absorption:

The mechanism of zinc absorption is still unclear. *In vitro* studies have shown that the ileum has the greatest capacity to absorb zinc (Emes & Arthur, 1975), while *in vivo* studies with isolated intestinal loops have shown duodenum to be the preferred site for zinc absorption. The absorption of zinc is thought to be facilitated by a low molecular weight zinc-binding ligand. (Evans *et al*, 1975). Zinc can be absorbed against a concentration gradient. As shown in Fig.2**,** there is a flux of zinc from mucosa at all times (Cousins, 1979). Cousins has suggested that thionein, a sulfur-rich metal-binding protein plays a role in the regulation of the entry of dietary zinc from the mucosal cell into the body. Zinc that is neither incorporated into the cell functions nor trapped as metallothionein can be transferred to the serosal border and picked up by the portal blood stream. Albumin is thought to be the normal carrier protein for zinc in the portal stream (Smith & Cousins, 1980).

Zinc is absorbed more efficiently than iron. Unlike iron, the body excretes substantial amounts of zinc via intestinal secretions as a part of its homeostatic regulatory mechanism (Matseshe *et al*, 1980). The efficiency of zinc absorption is greater during deficiency and lower under conditions of excess zinc (Fairweather-Tait, 1988).

Functions:

Zinc is required for structural and functional integrity of more than 2000 transcription factors (Coleman, 1992) and 300 enzymes (Vallee & Falchuk, 1993), therefore, almost all signaling and metabolic pathways are in someway dependant on at least one of the several zinc- requiring proteins. The functions of zinc can broadly be grouped as shown in Fig. 3. Most biochemical roles of zinc reflect its involvement in a large number of enzymes or as a stabilizer of the molecular structure of sub cellular constituents and membranes. Zinc participates in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids. It has recently been shown to play an essential role in the processes of genetic stability and gene expression in many ways including the structure of chromatin, the replication of DNA and transcription of RNA through the activity of transcription factors and RNA and DNA polymerases, as well as playing a role in DNA repair and programmed cell death (Falchuk, 1998; King & Keen, 1999; Berg & Shi, 1996). Its involvement in such fundamental activities probably accounts for the essentiality of zinc in all forms of life. Another important function of zinc is its participation in the antioxidant defense system, being a cofactor of superoxide dismutase.

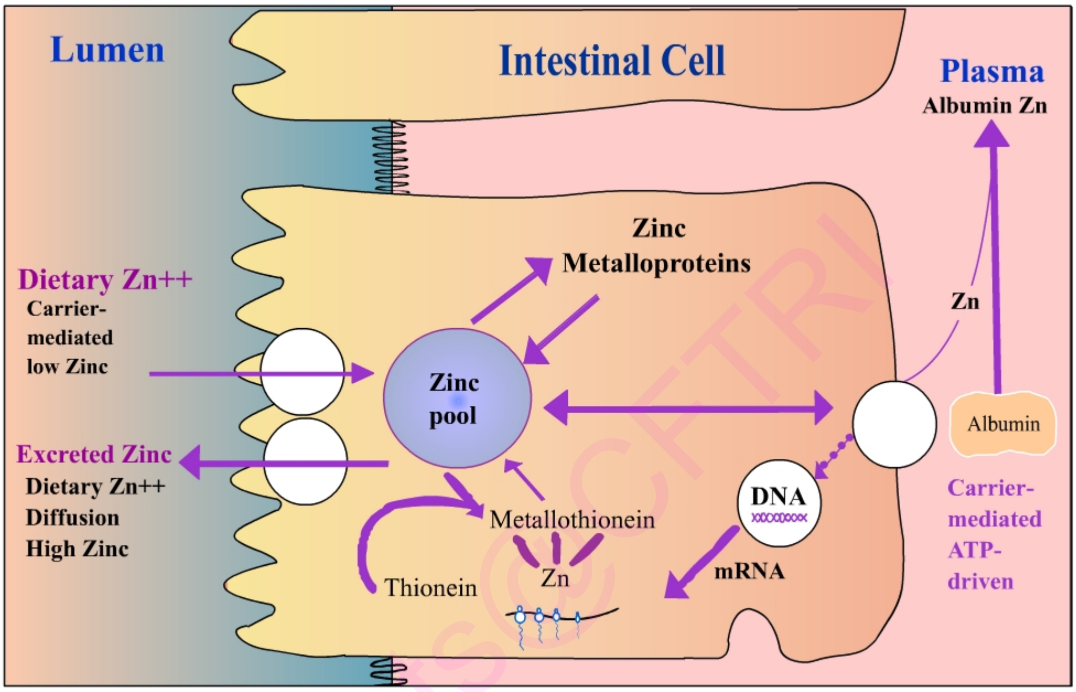


Fig.2 A model for zinc absorption showing the relationship between metallothionein and cysteine rich intestinal protein.

Source: Cousins, 1979

Fig. 3 Multiple Functions of Zinc

The requirement of zinc for growth and cell division is well established and several studies have suggested that DNA synthesis and cell division are more susceptible to deficiency of zinc than is protein synthesis (Chesters, 1989).

Zinc deficiency:

It is estimated that zinc deficiency affects one-third of the world's population and is the major factor contributing to 1.4 % of all the deaths worldwide (Table-3) (WHO, 2002). Although acute deficiency in developed countries is rare, marginal deficiency is thought to be relatively common (Hambidge, 2000), especially at early stages of the life cycle (infancy and childhood) when zinc requirements are relatively high. Marginal zinc deficiency and suboptimal zinc status have been recognized in many population groups in both less developed & industrialized countries. Although the assessment of zinc status is a complicated task, supplementation or fortification with zinc has been associated with increased linear growth, reduction in diarrhoeal disease, enhanced immune function and improved pregnancy outcome. Although the cause of suboptimal zinc status in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are likely the most common causative factors.

The essentiality of zinc was first documented in 1930s for growth and survival of animals (Todd *et al*, 1934). In 1960s it was understood that deficiency of zinc would lead to growth retardation, male hypogonadism, skin changes, and susceptibility to infectious diseases in humans (Prasad *et al*, 1961). It was believed that this nutrient deficiency was relatively rare and not until the 1990's was it recognized that marginal zinc deficiency is common and most likely a major nutritional problem worldwide. (WHO, 1996; Brown *et al*, 2002). Low zinc status has been correlated with a plethora of diseases (Table-4). Major impacts of zinc deficiency include reduced growth and suppression of immune function (Hambidge, 2000). Zinc deficiency during pregnancy affects the outcome of pregnancy. A high prevalence of zinc deficiency (55.5 %) has been reported among pregnant women. Severe zinc deficiency in animals has been associated with structural malformations of the brain such as anencephaly, microcephaly and hydrocephaly, with reduced activity, deficit in short term memory and spatial learning. In humans severe zinc deficiency causes abnormal cerebral function and impairs behavioral and emotional responses (Prasad, 1981). In children neuropsychological functioning activity or motor development is impaired (Black, 1998).

Table-3 Global Prevalence of "estimated" Zinc deficiency

Region	W Eur	N Am	E Eur	W Pac	L Am	China	SE Asia	S-S Africa	NE Africa	S Asia	World
Zn mg/d	3.2	2.9	2.1	2.0	1.5	1.5	1.1	1.0	1.0	0.8	1.5
% at risk	8.0	0.9	12.8	18.6	45.8	21.4	71.2	68.0	73.5	95.4	48.9

Source: WHO, (2002a)

Table-4 Manifestations of zinc deficiency

Growth retardation	Abnormal dark adaptation
Delayed wound healing	Weight loss
Hypogonadism	Oligospermia
Intercurrent infections	Anorexia
Altered immune response	Diarrohea
Increased abortion risk	Alopecia
Complications during delivery	Mental lethargy
Neural tube defects of foetus	Skin changes
Impotence, infertility	Hypogeusia
Taste abnormalities	Hyposmia
Emotional disorders	Hyperammonaemia
Amenorrhea	Growth retardation
Malabsorption syndromes	Inflammatory bowel disease
Impaired glucose tolerance	Rheumatoid arthritis
Reduced appetite	Night blindness
Delayed sexual maturation	Dandruff
Virtually all-dermatological disorders	
Connective tissue disease	
Frequent and/or severe infections	
Sleep and behavioral disturbance	

Source: Salgueria *et al*, 2000

Dietary sources:

Organs and flesh of mammals, fowl, fish, and crustaceans are the richest sources of zinc and provide a larger portion of absorbable zinc in the absence of any phytate. Eggs and dairy products are the next better sources of zinc. Most cereals and legumes have moderate levels of zinc, but their high phytate content reduces the amount of zinc available for absorption. When these staples are fermented (as in leavened breads and porridges prepared from fermented cereals). The fermenting organisms produce phytase that breakdown phytates, thus increasing the amount of absorbable zinc. Rice and starchy roots and tubers have lower zinc contents than legumes and cereals other than rice. For the most part, fruits and vegetables are not rich sources of zinc, although some green leafy vegetables have a fairly high zinc density. The zinc content (mg/kg) of various foods is: red meat and cheese (30–50) pulses/legumes (25–35) and whole grains (30–50) (Brown *et al*, 2001).

***Recommended Dietary Allowance*:**

The WHO committee estimated the physiological zinc requirements of adults as the sum of the amounts needed for tissue growth, maintenance, metabolism, and replacement of endogenous losses (WHO, 1996). Estimates of the physiological requirement for zinc are complicated by several factors including the absence of adequate epidemiological evidence on which to base an estimate and the difficulty in establishing estimates using a factorial technique. This latter problem arises because of the profound ability of humans to reduce their endogenous zinc losses and to enhance absorptive capacity as the dietary zinc intake becomes limiting. Added to this is the complication that the bioavailability of zinc from different diets ranges from around 15 to 55% (WHO, 1996). Nevertheless, a series of requirement estimates for zinc for adults have been set by WHO (WHO, 1996) which range from 5.6 to 18.7 mg per day depending on the bioavailability of zinc in the diet. The Indian Council of Medical Research has proposed estimated daily requirements of 5 mg for infants, 10 mg for children and 15 mg for adolescents and adults.

Bioavailability of iron and zinc from foods:

Bioavailability is the portion of a nutrient in food, which is absorbed and utilized. Utilization is the process of transport, cellular assimilation and conversion to a biologically active form(s) (O'Dell, 1984). 'Bioavailability' has been defined differently as follows:

- Mineral bioavailability is the measure of the proportion of the total mineral in a food or diet that is digested, absorbed and metabolized by normal pathways. (Fairweather- Tait, 1987).
- The efficiency with which consumed nutrients are absorbed from the alimentary tract and are thus available for storage or use (Forbes & Erdman, 1983; Bender, 1989).
- The 'degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal' (Ammerman *et al*, 1995)
- Bioavailability refers to that proportion of the total amount of a mineral element present in a nutrient medium that is potentially absorbable in a metabolically active form. The term `potentially absorbable' is used because the actual amount absorbed may be affected by numerous factors (Welch & House, 1984).
- Bioavailability represents the response of the test subject (human, animal, cells in culture, etc.) to the diet or food (Fairweather-Tait, 1987; Southgate, 1989).
- Recently, bioavailability has been redefined as meaning the fraction of an ingested nutrient available for utilization in normal physiological functions and storage (West & Eilander, 2001).

It is generally accepted that only soluble minerals can be absorbed. The fraction of the total mineral in the food or diet that is available for uptake by the intestinal brush border cell membranes is known as bioaccessibility, and is the first step in the absorption process (Salovaara et al, 2002), and therefore for the bioavailability that includes also absorption, metabolization and utilization for normal functions (Fairweather-Tait, 1992).

Quantitation of bioavailability:

Bioavailability of nutrients can be studied directly by employing humans or animals (*in vivo* methods) or by simulating the *in-vivo* situations (*in vitro* methods).

In vivo methods:

Metabolic balance technique:

In this approach, the amount of iron / zinc in the stools is subtracted from the iron / zinc in the diet consumed over a fixed period of time and the apparent absorption is calculated. The first attempts to measure the bioavailability of iron in the whole diet were made with chemical balance method (Moore & Dubach, 1951), but it has many drawbacks:

1. This method is tedious, time consuming and expensive.
2. It does not consider the immediate origin of the nutrient in the feces, but gives a useful overview of the nutrient balance.
3. It does not make correction for endogenous loss by way of the intestines. The biliary, pancreatic and intestinal secretions to the fecal output cannot be distinguished from that originating in the diet. With reference to Zinc, the normal contribution of endogenous zinc to the fecal pool is large and variable that true absorption of dietary zinc is difficult to determine (Solomons, 1982).
4. It can be used to study only whole meals but not individual meals or foods.
5. Small errors in estimating intake or excretion that are not well absorbed lead to large errors in calculating the balance figures.
6. Sufficient time should be allowed for the body to adapt to the new diet.

Rate of repletion:

This is another method to estimate bioavailability of nutrients. In this method, the rate at which a depleted organism gets repleted by different sources of the element under study is measured. The test materials are usually compared with a well-absorbed reference salt and the results are expressed as the ratio between the two. This method is useful to investigate the efficacy of different sources of an element in treating trace element deficiency.

Iron bioavailability can be studied by a standardized hemoglobin repletion method proposed by Fritz *et al* (1975). This method involves rendering the experimental animals anemic by feeding them a diet deficient in iron, followed by feeding the test foods containing iron. Haemoglobin concentration is measured during the repletion period. The results obtained by this method are relative rather than absolute, but, the draw back of this method is that it is impracticable in human subjects (Solomons, 1982), nor can the results obtained by the animal study be extrapolated to the human situation.

Plasma Appearance / Circulating zinc response:

In this method, the appearance of an element in plasma after its oral ingestion is measured. The source should be highly concentrated and must be sufficient enough to create an observable plasma tolerance curve, i.e. plasma appearance must exceed disappearance over the period of absorption from the gut. The disadvantage of this method is that it is not applicable to most food sources of trace elements.

Measurement of growth rate:

This can be employed when growing animals are used and the element under study is the only limiting factor.

Isotope Techniques:

The metabolic fate of minerals in a specific days' diet, a specific meal or a food can be distinguished from the same minerals from other sources by using isotopic tracers, either radio or stable isotopes. The isotopes of zinc are of atomic mass 64, 65, 66, 67, 68, 69,70 and 71. $Zinc^{65}$ is a radio isotope with a half life of 244 days, which can be used for experiments but it exposes the subject to long durations of radiation.

Radio isotope techniques:

Radioactive isotopes have been used widely to study absorption and metabolism of trace elements in man and animals. Radio labeling is done by two ways- intrinsic and extrinsic labeling. Intrinsic labeling involves addition of labeled isotopes in the media where the plants are grown or in the feed in case of animals. The labeled foods are then fed to the subjects and the bioavailability is studied by monitoring the isotope (Swanson *et al*, 1983). Extrinsic labeling involves spiking a meal with tracer quantity of labeled salt in a solution and feeding the meal to the volunteers on an empty stomach, after an overnight fast, followed by whole body counting immediately after the meal, as well as 12-14 days later. The body retention of iron / zinc can also be estimated by measuring the circulating blood (Cook *et al*, 1972).

Over the decades, reliable data has been generated and studies in India by Rao *et al* (1983) compared chemical balance method with extrinsic tag method and found that the chemical balance method gave three times higher values for iron absorption than those obtained by extrinsic tagging technique. Radioisotopes can be used for balance technique; the absorption of an isotope can be measured by deducting fecal and urinary excretion from the administered dose over the appropriate time period. Measures of retention can be equated with absorption when there is negligible excretion of absorbed isotope over the experimental period. This is true only in case of iron but in the case of zinc, measures of retention always underestimate absorption especially when the subjects are consuming a high zinc diet and excreting large quantities of endogenous zinc in the feces (King & Turnland,1989).

Plasma appearance of an isotope from a labeled food can be used to indicate trace element bioavailability. The rate of absorption or retention can also be studied, which is applicable only to iron as about 80 % of absorbed iron is utilized. The enrichment of RBC with radio-iron can be used to assess dietary iron bioavailability (Fairweather-Tait, 1992; Bothwell *et al*, 1979).

Radio labeling gives a reliable estimate of true absorption. By using isotopic tracers, the metabolic fate of minerals from the food, diet or a specific meal can be distinguished from other sources. The difference between heme iron and non-heme iron bioavailability was established using radioisotope tracers. But it is expensive, time consuming and requires sophisticated equipment. It has the disadvantage of having a radioactive half-life apart from being biohazardous.

Stable isotopic techniques:

Stable isotopes are naturally occurring nuclides of element with same atomic number but differing numbers of neutrons. They have similar chemical properties but differ in mass and, unlike radioisotopes, pose no known hazards to experimental subjects. Stable isotopes were first used as a tracer of mineral in 1963 to measure plasma clearance of iron by using Fe^{58}, (a stable isotope of iron) (Lawman & Krivit, 1963). Instrumentation facilities such as Inductively Coupled Plasma Emission-Mass Spectrometry (ICP –EMS), Fast-atom-bombardment mass spectrometry (FAB / MS), and Thermal ionization mass spectrometry have made studies with stable isotopes possible. Like radioisotopes, stable isotopes are added to foods or diets of interest and determined by fecal monitoring. Multiple isotopes of the same element, as well as multiple elements can be used simultaneously (Turnland, 1991). Many studies are being carried out using stable isotopes to study bioavailability of minerals from foods and also to identify the various factors that influence their absorption.

***In vitro* Techniques:**

In order to estimate mineral bioavailability, several *in vitro* methods have been proposed as an alternative to *in vivo* methods. Most of these *in vitro* methods consist of a simulation of gastrointestinal digestion followed by a determination of how much of the element is soluble, or by dialysis through a membrane of a certain pore size. In fact, at this level, it is the bioaccessibility that is measured, and the values obtained can be used to establish trends in bioavailability, or relative bioavailability (Wienk et al, 1999).

The earlier *in vitro* techniques involved measurement of soluble or ionizable fraction of the mineral after simulated gastrointestinal digestion (Rao & Prabhavathi, 1978). An improvement of these methods involves measurement of the ionizable fraction of the mineral by equilibrium dialysis (Miller *et al*, 1981). The mineral fraction that dialyzes across a semi permeable membrane of a certain pore size during simulated gastrointestinal digestion is considered as the fraction that is available for absorption (bioaccessible fraction). This dialyzability method was further validated by Luten *et al* (1996), by carrying out inter laboratory trials.

Absorption studies using cell lines:

A new *in vitro* Caco-2 cell system has been developed for predicting iron bioavailability from food (Garcia *et al*, 1996) and meal digests (Glahn *et al*. 1996). It combines the simulation of gastrointestinal digestion as in the method of Miller *et al*, with nutrient uptake by Caco-2 cells, a human intestinal epithelial cell line. This method involves subjecting food samples labeled with ^{59}Fe to simulated gastrointestinal digestion provided for equilibrium dialysis to obtain the dialyzable fraction of the mineral. In the next step, the dialyzable fraction is incubated with Caco-2 cell monolayers. The absorbed mineral fraction is quantitated by measuring the radio activity present in the surface of the monolayer, after a specific time interval. Thus, this *in vitro* model measures iron absorbability from foods by quantitating

Caco-2 cell ferritin formed after iron uptake. This method can also be employed without the use of radio labeled iron. This method provides for assessment of the bioavailable fraction of the mineral, which is of more practical value than the bioaccessible fraction.

In vitro methods offer a more practical alternative to *in vivo* studies involving human and animal models. These methods measure the amount of mineral available in the gastrointestinal tract for absorption (bioaccessibility) (Salovaara *et al*, 2002). These methods are widely used because of their satisfactory correlation with *in vivo* studies (Roig *et al*, 1999; Wienk *et al*,1999). Bioaccessibility values must be taken as relative indices of bioavailability, which means that the method used provides a good basis for establishing tendencies, comparisons and the determination of effects caused by different factors (Azenha & Vascondecelos, 2000). *In vitro* models allow us the possibility to control the conditions of the assay optimally, which may lead to a high precision, with lower variability, than that of the *in vivo* methods. Other advantages are the low cost and shorter time needed to obtain results than with *in vivo* methods. It is, however, important to stress that the results from *in vitro* methods are relative compared to absolute estimates of mineral bioaccessibility or bioavailability, because they do not take into account the physiological factors that can affect bioavailability. They are however useful for comparing and / or classifying foods according to the bioaccessibility of a certain mineral.

Factors influencing iron and zinc absorption:

Trace element absorption is influenced by a number of dietary factors, which include both inhibitors and enhancers of absorption (Table-5).

Influencers of iron absorption:

Bioavailability of heme and non-heme iron differ, due to differences in the mechanism of their absorption. In humans, the efficiency of absorption of non-heme iron is lower than that of heme iron (Bothwell *et al.*, 1979). The bioavailability of non-heme iron in iron-replete people ranges from 2% to 20%, while that of heme iron ranges from 15% to 35% (Carpenter & Mahoney, 1992). The absorption of non-heme iron is influenced significantly by dietary composition and iron status (physiological

Table-5 Influencers of the absorption of trace elements

1. Diet composition

- Protein quality (protein source - animal / plant, amino acid balance)
- Protein quantity
- Trace element concentration
- Physiochemical form of trace element
- Nutrient interactions (element-element; element-organic compounds)
- Promoters (meat, ascorbate, citrate, vitamin D, some amino acids, some sugars)
- Inhibitors (phytate, oxalate, polyphenols, fiber)

2. Food processing

- Cooking
- Fermentation
- Milling
- Malting
- Germination
- Soaking

3. Host-related factors

- Age
- Sex
- Physiological status (pregnancy, lactation, physical activity
- Nutritional status (moderate or frank deficiency, lean body mass)
- Disease (including parasitism)

Source : House, 1999

iron stores) (Carpenter & Mahoney, 1992), while it was found that iron status does not have a marked effect on the absorption of heme iron (Fairweather-Tait, 1992). In contrast, Hallberg *et al*. (1997) reported that absorption of both heme and non-heme iron were influenced by iron status, this effect being more pronounced in the case of non-heme iron.

Bioavailability of iron from food systems is an outcome/resultant of the interaction of its components. Studies have shown that the absorption of iron from typical Indian meals varies from about 2-10% (Rao, *et al*. 1983)

Heme iron associated with meat, poultry or fish was found to be highly bioavailable and the only dietary constituent known to influence its absorption appears to be meat. An enhancing effect of meat and fish on heme iron absorption was first reported by Layrisse *et al* (1968), and subsequently confirmed by many other researchers (Cook & Monsen, 1976; Morris & Ellis, 1983; Hallberg *et al*, 1978; Layrisse & Torres, 1972). Beef and ascorbic acid enhanced iron uptake by Caco-2 cells; it has been suggested that dialyzable factor(s) < 14,000 Daltons released during beef digestion, were responsible for the iron absorption-enhancing properties of beef (Gangloff *et al*, 1996).

The absorption of non-heme iron is influenced by several dietary constituents such as phytic acid, polyphenols, organic acids, dietary fiber etc. Phytic acid (Myoinositol hexaphosphate) is the main storage form of phosphorus in cereals and legumes. In seeds it is located in the endosperm. The Phytic acid content of these food grains varies between 0.5 and 6%, and accounts for 60-90% of the total phosphorous content of the grain (Fox & Tao, 1989). The phytic acid molecule can be dephosphorylated by means of enzymes or as result of high temperature processing to yield myoinositol bi-, tri-, tetra- and pentaphosphates (Fox & Tao,1989; Lonnerdal *et al*, 1989). Phytic acid retains minerals in the intestinal lumen by forming insoluble complexes. The metal complexes of phytic acid and lower inositol phosphates are poorly soluble at the pH of gastrointestinal tract and may reduce the bioavailability of minerals such as iron zinc, calcium and copper (Torre *et al*, 1991). The affinity of inositol tri- and tetraphosphates for mineral elements is lower than that of inositol hexaphosphate, and the solubility of metal complexes formed with the former two phosphates is higher than with the hexaphosphate (Persson *et al*, 1998). The decreasing order of metal complexation with phytic acid has been reported to be $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$ at pH 7.4.

In vitro studies have indicated that both chemical forms of iron present in foods, namely, Fe^{++} and Fe^{+++} strongly interact with phytate at any pH, thus being rendered unavailable for absorption (Nolan *et al*, 1987). Ferrous and Ferric iron also showed strong interaction with protein-phytate complex, which negatively influenced iron availability (Champagne *et al*, 1985). But the study by Reddy *et al* (1996) has shown that effect of phytate on Fe absorption does not depend on the protein composition of the test meals.

In vivo studies carried out in human beings indicate that inhibition of iron absorption is strongly related to the amount of phytic acid present in the diet (Brune *et al*, 1984, Hallberg *et al*, 1989). However, other studies have shown that dietary phytate had little effect on the bioavailability of Fe in either rats (Akhtar *et al*, 1987) or humans (Beard *et al*, 1988), and monoferric phytate from wheat bran was highly available to rats (Morris & Ellis, 1976) and to humans (Hallberg *et al*, 1987). Sodium phytate decreases iron absorption in man (Hallberg & Solvell, 1967). The poorer absorption of iron from brown bread compared with white bread has been attributed to the high content of phytate in brown bread (Moore, 1968).

The influence of dietary fibre on the absorption of iron is unclear. Fibre from different plant sources has been shown to have different chemical structures and thus, different binding capacities (Van Soest & Jones, 1988). *In vivo* studies indicated that both ferrous and ferric iron is retained by fibre in the intestinal lumen due to the formation of poorly soluble fibre - mineral polymers. *In vitro* studies have indicated that neutral detergent fibre and the acid detergent fibre affected the absorption of iron (Platt & Clydesdale 1987). Both *in vivo* and *in vitro* studies have shown that among the various components of dietary fibre, cellulose poorly binds iron, whereas hemicellulose, lignin and pectin strongly bind iron rendering it unavailable for absorption (Rossander, 1987; Platt & Clydesdale, 1987). Sugar beet fiber fed to rats enhanced iron absorption (Fairweather-Tait & Wright, 1990), and pectin with a high degree of esterification and low molecular weight improved iron absorption in rats (Kim & Atallah, 1993; Kim *et al*, 1996). The effects of dietary fibre on the bioavailability of trace elements appear to be confounded by the simultaneous presence of phytate (Brune *et al*, 1992; Gibson, 1994).

Polyphenols present in tea are reported to markedly reduce the absorption of iron from foods. The absorption from bread was reduced to one third and from a vegetable soup to one fourth when served with tea (Disler *et al*, 1975). In a study on the effect of various drinks on the absorption of non-heme iron from a hamburger meal, tea and coffee (sources of tannin) reduced the absorption by 61 and 33%, respectively, this effect being ascribed to the formation of insoluble iron - tannin complexes in the intestinal tract (Conrad & Crosby, 1962). Tannins might be partly responsible for the low bioavailability of iron in many vegetable foods (Disler *et al*, 1975). Additionally, tannins may also indirectly influence iron absorption by interfering with protein digestion and absorption; as a result, undigested proteins and peptides in the intestinal lumen may in turn bind iron irreversibly, rendering it unabsorbable (Deshpande *et al*, 1984; Shahkalili *et al*, 1990).

Ascorbic acid has been found to influence iron absorption, especially non-heme iron. In one of the early studies orange juice with a high content of ascorbic acid markedly increased non-heme iron absorption in a dose dependent manner, while there was no effect on the absorption of heme iron (Cook *et al*, 1972; Sayers *et al*, 1973; Hallberg & Solvell, 1967). The addition of cauliflower, which contains about 70 mg of ascorbic acid to a vegetarian meal, increased the absorption of non-heme iron three times. Ascorbic acid, either in the crystalline form or inherent in food, has the same promoting effect on non-heme iron absorption (Hallberg, 1981; Heinrich, 1987). The effect ascorbic acid seems to be independent of the effect of other promoters of iron absorption, such as meat. In the presence of an inhibitor of non-heme iron absorption such as tea, the relative enhancing effect of ascorbic acid on iron absorption seems to be the same. The absolute increase in amount of iron absorbed, however, is less because of the lower original absorption (Disler *et al*, 1975; Rossander *et al*, 1979). The enhancing effect of ascorbic acid on food iron absorption is attributed to its reducing ferric to ferrous iron, which is more soluble at the pH of the upper regions of the intestinal tract (Carpenter & Mahoney, 1992). In addition, in the highly acidic conditions in the stomach, ascorbate may form a chelate with ferric iron, which remains stable at the more alkaline pH in the intestine (Bothwell *et al*, 1989).

The other dietary organic acids, including citric, malic, tartaric and lactic acid, enhance iron absorption (Bothwell *et al*, 1989). The enhancing effects of ascorbic acid and citric acid on iron absorption appear to be additive, which may account for the relatively high bioavailability of dietary iron when it is consumed along with citrus fruits and juices (Ballot *et al*, 1987). An *in vitro* study suggested that citric acid rather than ascorbic acid may be the major enhancer of iron availability from many fruits and vegetables (Hazell & Johnson, 1987).

In infancy, lactoferrin, an iron-binding protein in human milk, promotes the absorption of iron through lactoferrin receptors on the surface of the intestinal mucosa of infants. This may explain why the small amount of iron that is present in milk is well absorbed by breast fed infants (Berdnaier, 1998).

Vitamin A, a fat-soluble vitamin also has an influence on the absorption of iron. In earlier studies with rats, vitamin A affected the distribution of iron in the body but did not affect iron absorption (Mejia *et al*, 1979). Studies with mice indicated that vitamin A may increase alimentary absorption of iron and promote the mobilization of iron from storage sites (Hong *et al*, 1994). Recent studies indicated that supplemental vitamin A overcomes the depressing effect of phytate and polyphenols on iron absorption, apparently, by binding iron during the digestive process and thereby keeping it in a soluble form as a vitamin A-iron complex (Layrisse *et al,* 1997). Supplemental vitamin A reversed the anemia that developed in Vitamin A deficient individuals (Mejia & Chew, 1988).

The absorption of iron, both heme as well as non-heme, is affected by other factors such as the physiological status of the animal or person that consumes it. Low iron status, anemia, pregnancy and growth phase increase the iron absorption from food. Certain conditions like achlorohydria and increased motility decreases the iron absorption (Zhang *et al*, 1991).

Influencers of zinc absorption:

Early observations in rats and chicks suggested that zinc from foods of animal origin were more bioavailable than zinc from plant origin (O'Dell & Savage, 1972; Scott & Ziegler, 1963). Further research on zinc bioavailability indicated that plant foods have certain inhibiting factors like phytic acid, calcium, iron, dietary fiber etc. which affect the bioavailability of zinc.

Forbes and Yohe (1960) suggested that phytic acid inhibits zinc absorption. It was speculated that the dietary basis for the zinc deficiency in the Middle East was the high phytate content of the diet (Prasad *et al*, 1963). 'Tanok', a rural diet that is made from whole wheat, reduced zinc absorption (Reinhold *et al*, 1976). However the relative and absolute impact of phytic acid on zinc absorption is still not clear.

Several studies on the influence of phytic acid on zinc absorption revealed that it reduced zinc absorption (Reinhold *et al*, 1973; Obizoba, 1981). Reduction in the availability of zinc in Textured Vegetable Protein was attributed to its phytic acid content (Davies & Olpin, 1979). However, Ellis *et al* (1981) found no difference in zinc absorption in adult men who consumed varying concentrations of phytic acid. Phytate from an alternative dietary supplement had no effect on the zinc status of undernourished rats. Siqueira *et al* (2001) concluded that in mature peas phytic acid was not solely responsible for the decrease in availability of zinc to rats. Treatment with phytase and leavening of bread increased zinc availability *in vitro* due to degradation of phytate (Reinhold *et al*, 1974; Davies & Nightingale, 1975). A recent study by James *et al* (2005) suggests that addition of phytase, a phytate degrading enzyme, to foods improves zinc status, with consequent increases in body weight, and stronger bones, thus qualifying for food supplementation.

Studies have indicated that varying zinc / phytic acid ratios altered zinc availability. (Oberleas & Prasad, 1975; Davies & Olpin, 1979; Lo *et al*, 1981). Ratios equal to or greater than 12.5:1 reduced the availability of zinc. However, studies carried out by Morris and Ellis (1980) suggested that this ratio is not a determinant of zinc availability. The WHO included the phytate : zinc ratio of the food as criteria for categorizing diets according to the potential availability of their zinc content (WHO,1996). Diets have thus been categorized as low, moderate, or high with respect to zinc bioavailability. Diets with low availability of zinc may contain high phytate, soybean-protein products or have a phytate: zinc molar ratio >15.

The influence of dietary fiber on zinc availability is as uncertain as its influence on iron bioavailability. An animal study on zinc absorption and balance found that zinc absorption was not affected by cereals (rich in dietary fibre) and Neutral Detergent Fibre. Addition of various types of bran to the diet did not affect zinc balance (Guthrie & Robinson, 1978; Sandstead *et al*, 1978; Greger *et al*, 1978). Components of dietary fiber like cellulose and hemicellulose had a negative influence on the bioavailability of zinc (Reinhold *et al*, 1976; Drews *et al*, 1979); but the studies of Behall *et al* (1987) indicate that cellulose and carboxymethyl cellulose addition did not significantly affect zinc balance.

In a high protein diet, 45% lactalbumin significantly increased the absorption of zinc and its deposition in bone as compared to 15% lactalbumin (O'Dell, 1984). A negative zinc balance was observed with 14 mg soy protein diet, while 12 mg of protein from animal source resulted in a positive balance (Cossack & Prasad, 1983). Supplemental sulfur-containing amino acids increased zinc bioavailability in rats fed either soy protein (Greger & Mulvaney, 1985; House *et al*, 1997) or lactalbumin (Snedeker & Greger, 1983). The bioavailability of Zn has been associated positively with dietary methionine (Hunt *et al*, 1987).

Calcium is another factor found to influence the availability of zinc. The detrimental effect of high calcium is dependant upon the presence of phytate in the diet (O'Dell, 1979). Calcium, when given in diets with soy proteins lowered zinc bioavailability by 50 % while it had little or no effect when egg white supplied the protein. This is because soy proteins are rich in phytates. The detrimental effect of phytate is accentuated by high levels of dietary calcium. It has been suggested that any possible negative effect of calcium on zinc bioavailability in meals rich in phytic acid is overcome by concomitant increase in animal protein, if the calcium is supplied by milk (Forbes *et al*, 1983; Davies & Olpin, 1979; Morris & Ellis, 1980; Navert & Sandstrom, 1985).

Phytate X calcium / zinc ratio may be a useful index of the bioavailability of dietary zinc, in view of the synergetic influence of calcium on the inhibitory role of phytate in zinc absorption (Oberleas & Harland, 1981; Forbes *et al*, 1983). *In vivo* zinc availability is a dynamic property that depends on both the amount of zinc and its source in the meal, as well as on the interactions of zinc with other components within the meal.

Zinc absorption seems to be influenced not only by phytic acid - calcium complex alone, but also in association with dietary fibre. Combination of high levels of dietary fibre, calcium and phytic acid together with low intakes of available zinc accounted for high incidence of low serum zinc levels. Approximately 75% of the phytic acid is associated with the soluble fiber fraction. Studies reported on the effect of cereal fibers on zinc bioavailability in humans found that zinc absorption is inversely related to the phytic acid content of the meal (Frolich & Asp, 1980, Frolich & Nyman, 1988; Sandstrom *et al*, 1987; Farah *et al*, 1984). Reinhold *et al* (1976) opined that in cereal-based foods rich in both phytic acid and fiber, fiber could be a major factor affecting zinc bioavailability.

Feeding chitosan, alginic acid or raw potato starch, as dietary fiber polysaccharides lowered the inhibitory effect of phytic acid on zinc bioavailability (Yonekar & Suzuki, 2003). Thus it can be concluded that phytate and dietary fiber occur together in plant foods, and that both contribute to the reduction of zinc bioavailability in synergy.

The effect of oxalic acid on zinc bioavailability is unclear, as there are contradictory results. In one study zinc availability was unaffected by oxalate (Welch *et al*, 1977), while in another foods rich in both oxalic acid and fiber reduced zinc balance (Kelsay, 1983). Folic acid was found to be associated with decreased availability of zinc in some studies (Ghishan *et al*, 1986), but not in others (Keating *et al*, 1987).

Effect of food processing on bioavailability of iron and zinc:

Most foods are consumed after they are subjected to processing. Simple household processing methods like cooking, soaking, fermenting, germination and baking help in the digestion of food and facilitate availability of nutrients apart from improving palatability. The anti-nutritional factors are also reduced thus enhancing the availability of nutrients. Food processing aids in the bioavailability of trace metals in foods by reducing the amount of dietary phytate (Sandstrom, 1989; Lei *et al*, 1993; Svanberg *et al*, 1993; Schlemmer *et al,* 1995; Larsson *et al,* 1996; Harland & Morris, 1995).

Heat processing:

Heat processing involves subjecting the food to heat treatment like boiling pressure-cooking, microwave cooking etc. Boiling of rice and pulse and frying whole wheat on the girdle did not affect solubility of iron. Parboiling of rice lowered the availability of iron (Prabhavathi & Rao, 1979). Mild heat treatment increased the digestibility of most foods and appeared to reduce the phytic acid contents of tubers, but not of cereals and legumes (Marfo *et al,* 1990). Cooking methods improved the HCl-extractability of zinc by pressure-cooking and ordinary cooking of soaked dehulled pigeon pea seeds as well as rice beans (Duhan *et al,* 2004).

Soaking:

Soaking is a pretreatment in the process of fermentation and germination of food grains, which might reduce phytic acid content. Studies have shown that soaking leads to a reduction in phytate content, thus enhancing mineral HCl-extractability, or in other words, mineral bioavailability (Duhan *et al,* 2002; Sandberg & Svanberg, 1991). Soaking of grains did not increase the solubility of iron (Prabhavathi & Rao, 1979).

The effects of soaking whole cereals (maize, finger millet, rice and sorghum), and legume seeds (mung bean, cowpea, soybean) on iron, zinc and phytate contents indicated that soaking whole seeds for 24h led to the leaching of iron and, to a lesser extent, of zinc ions into the soaking medium. Soaking also resulted in a significant reduction in the phytate content of finger millet, maize, rice and soybean, but did not improve the phytate / iron molar ratio, while decreasing the phytate / zinc molar ratio only slightly. Soaking on its own was not found to be a good method for improving mineral bioavailability but the results showed that, in combination with other treatments, or with optimized soaking conditions, it could nevertheless prove useful. It was found that no phytate remained in the soaking medium, so, in order to limit mineral loss, the water used for soaking can also be used for cooking food grains which absorb a considerable amount of cooking water and, in this way, the leached minerals may be to a certain extent recovered (Lestienne *et al*, 2005).

Germination:

Germination involves soaking the grain and allowing it to sprout in the absence of water. It is also a part of the malting process. Apart from other nutritional changes during germination, *de novo* synthesis and activation of endogenous phytases occurs in cereals and legumes (Cosgrove, 1980). Degradation of phytate, an inhibitor of mineral absorption, by phytase may result in increased bioavailability of micronutrients. It is found that metal binding capacity of degraded phytate (lower inositol phosphate) is much less (Agte *et al,* 1998). Increased zinc availability as a result of sprouting in whole rice bean, and increased iron solubility after germination of green gram and wheat (24h) and chickpea (48 h), have been evidenced (Kaur & Kawatra, 1999).

Malting:

Malting involves germination of the grain followed by drying by roasting immediately after sprouting. Malting and soaking in the processing of oats resulted in an improvement in zinc and iron absorption from the resultant products (Larsson *et al,* 1996).

Fermentation:

Fermentation by microorganisms is encountered in the preparation of certain food products. Fermentation improves the digestibility of foods, and availability of nutrients. Various studies on zinc and iron bioavailability from bread, a fermented product, have shown that there is an increase in their availability. The ionizable iron content of bread was higher than that of '*phulka*' (unleavened bread),indicating that the process of leavening (fermentation) reduced the phytic acid content of the dough (Reinhold *et al,* 1974; Davies & Nightingale, 1975; Lopez *et al*, 2003; Navert & Sandstrom,1985). Iron solubility was not improved as a result of fermentation in other products like '*idli*' (Prabhavathi & Rao, 1979).

Interactions of trace elements:

Biologically important interactions between chemically similar metal ions were predicted in 1970. They often exhibit a biological competition, at the level of the intestinal mucosa when co-ingested (Hill & Matrone, 1970).

Fe-Zn interaction:

Both iron and zinc are transition elements having identical outer electron shell configurations. In 1971, the question of the dietary interaction of iron and zinc was first addressed in metabolic studies of nine young women. When the diet provided Fe / Zn ratio = 2, no competitive interaction was demonstrable between the two elements (White & Gynne, 1971). Studies carried out subsequently inferred that zinc absorption reduced with increasing concentrations of inorganic iron (Inglet, 1983).

Iron / zinc ratios ranging from 0:1 to 3:1 in solutions containing 25 mg of zinc and corresponding iron as ferrous sulphate produced a decrease in plasma zinc level (Johnson *et al*,1981), while heme iron at 3:1 ratio did not affect zinc uptake, indicating that the form of iron is an important determinant of the interaction (Payton *et al*,1982). Iron in the ferrous oxidation state is the more potent competing species for the uptake pathway of zinc (Solomons *et al*, 1983). These authors suggested that 25 mg of total ions (zinc and iron) administered as a single oral dose is the point of saturation where there is no interference in the adult intestinal tract.

It is increasingly recognized that iron and zinc deficiencies occurred together in various populations and may have to be addressed together. Combined treatment with iron and zinc has been proposed as one possible solution. However metabolic studies and supplementation trials suggest antagonistic relationship between zinc and iron, in which zinc reduces the positive effect of iron supplementation and *vice versa*. For example, when given to adults as solutions in ratios > 2:1 inorganic iron was found to compete for absorption with zinc (Solomons, 1986). Zinc absorption in fasting pregnant Peruvian women administered iron or iron + zinc was significantly reduced compared with non-supplemented women (*O'Brien et al*, 2000). In women administered only iron, plasma zinc concentrations were also lower, compared with controls. Conversely, there were smaller improvements in hemoglobin and serum ferritin concentrations in Indonesian children administered both iron and zinc than in children given iron alone (Schultink *et al*, 1997; Lind *et al*, 2003). Further evidence shows that excessive intake of iron as supplements or in formulated foods can impair zinc status in infants and in all age groups (Prasad *et al*, 1978; Walravens & Hambidge, 1976; Craig *et al*.1984, Hambidge *et al*, 1983, Campbell *et al*, 1985, Breskin *et al*, 1983).

But prenatal iron supplementation, which is commonly practiced with 60 mg per day in countries such as Nepal with high prevalence of iron deficiency anemia, did not appear to alter zinc status in the presence or absence of daily supplementation with 25 mg zinc. Among severely anemic women, there is some cause for concern regarding supplementation with zinc, as this may reduce the hematologic impact of treatment with iron. However, this needs to be confirmed in studies that include larger numbers of severely anemic women (Parul, 2001).

Although the results are conflicting, the negative effect of iron on zinc reported in some of the studies has stimulated concern over the possible adverse effects of fortifying foods with iron, as well as the practice of taking iron supplements, on zinc nutrition. One of the main problems with research on zinc metabolism is the lack of suitable and sensitive indices of body zinc levels.

Fe - Ca interaction:

The influence of calcium on iron is unclear. Heme and non-heme iron absorption was found to be inhibited by calcium by interfering with the transport of iron through the mucosal cell in humans (Olivares *et al*, 1997; Hallberg *et al*, 1993). Supplemental calcium as inorganic salts or as dairy products impaired the absorption of heme iron by humans (Hallberg *et al*, 1993); Doses below 40 mg of calcium had no effect; maximal inhibition of 50% to 60% occurred with up to 300 mg of calcium, with no further inhibition at higher amounts, suggesting an incomplete block of iron absorption, thus indicating that (1) calcium has a direct rather than an indirect effect on heme-iron absorption and counteracts the enhancing effect of meat on heme-iron absorption and (2) calcium appears to interfere with mucosal intracellular transport of iron along a part of the pathway that is common for heme-iron and non-heme–iron transport but not luminal as was thought earlier.

The effect of $CaCO_3$ supplementation (400 mg of calcium per meal) on the absorption of iron consumed concurrently was examined in healthy volunteers using stable iron isotopically labeled and fecal monitoring. $CaCO_3$ supplementation was shown to significantly reduce non-heme iron absorption. Iron nutrition may be improved by reducing the intake of dairy products with meals that provide most of the dietary iron and by redistributing the daily calcium intake to meals with minor iron content. It was also suggested that consuming dietary calcium at least 2 h before a meal did not affect the absorption of iron from an iron-containing meal (Hallberg *et al*, 1992; Gleerup *et al*, 1995).

A study conducted on women and girls in 6 European countries indicated that dietary calcium intake is weakly and inversely associated with the iron status of blood in girls and young women, irrespective of whether calcium was ingested simultaneously with iron (Van de Vijver et al, 1999).

A recent study (Reddy & Cook, 1997) indicated that dietary calcium did not significantly affect non-heme iron absorption from a varied diet. Another study carried out to study the influence of calcium on iron availability from infant foods with high calcium found that calcium did not influence iron absorption in infant piglets (Ine *et al*, 1999).

***Interactions with other minerals**:*

Limited studies have been carried out to see the effect of other minerals on iron and Zinc absorption. Magnesium did not affect zinc absorption tested at 0:1 and 3:1 Mg/Zn levels (Geders et.al, 1981). Phosphorus has been shown in animal studies to reduce iron absorption; however, studies in man indicated that a reduction is only observed when both calcium and phosphate are added (Monsen & Cook, 1976). Excess copper decreased zinc absorption from rat intestinal segments maintained *in situ* (Vancampen, 1970) and Cadmium, Lead, and Tin interacted with zinc (O'Dell, 1984).

Scope of the present investigation

Cereals and legumes are staple foods in the diet of many populations, especially in developing countries, where they are the main source of energy and protein. These are also the main sources of minerals such as iron and zinc. Even though these food grains can substantially contribute to an adequate mineral intake, the mineral availability, especially as far as iron is concerned is generally poor, since it is affected mainly by phytic acid, tannin and dietary fibre components inherent in them. While the effects of these constituents on iron absorption have been the subject for numerous studies, little is known about the influence of the same on zinc availability. Even if net zinc intake from these staples appears adequate, compromised zinc status in populations dependent on these grains is common. It is therefore important to identify and evaluate dietary factors that interfere with zinc absorption. With such knowledge, strategies of avoiding or limiting components with inhibitory effects on zinc absorption and of choosing food or dietary components that enhance the same may be adopted. As such, a number of staple cereals and pulses commonly consumed in India have been examined in this investigation for zinc bioaccessibility value. These staple grains are generally consumed in the cooked form, and such a treatment may have a marked effect on the matrix and composition of food and therefore on iron and zinc availability. Hence, the bioaccessibility values of these two minerals have also been examined in heat- processed food grains.

Iron deficiency is often accompanied by other nutrient deficiencies such as zinc and copper deficiencies, especially when iron deficiency is caused by insufficient dietary intakes of micronutrients, as is often the case in developing countries. As a result, supplements containing iron and multiple trace elements and minerals are used by millions of people world-wide. It is important to provide detailed information regarding safe upper intake limits for supplements to minimize adverse effects on mineral absorption caused by mineral - mineral interactions. Iron supplements have been reported to decrease zinc absorption in pregnant women and lower serum zinc concentrations. Furthermore, a negative effect of iron provision through food fortification on zinc absorption could further exacerbate the zinc deficiency status commonly prevalent in populations dependent on plant foods. It is therefore important to practically evaluate any possible interactions between iron & zinc. Calcium has been shown to interfere with the absorption of non-heme and heme iron in a dose-dependent manner. The addition of milk or milk products to common meals brings the absorption of iron by as much as 50 - 60%. Effect of calcium on zinc availability at nutritional concentrations is reported to be less pronounced in human studies. However, a negative interaction of calcium with zinc absorption is possible when the former is consumed at supplementary levels. In this context the influence, if any, of exogenous iron and calcium on zinc bioaccessibility from selected food grains has also been assessed.

Domestic food processing such as germination and fermentation is known to affect the bioavailability of iron and zinc, probably by modulating the factors that act as promoters or inhibitors of mineral absorption. In this context, the much- practiced germination and fermentation of grains as encountered in Indian dietary have been examined for their influence on zinc and iron bioaccessibility.

Various organic acids that are present in foods, such as ascorbic, citric, malic & lactic acid have been shown to facilitate the absorption of iron. Use of food acidulants such as amchur, lime, tamarind and kokum is common in Indian dietary. The presence of organic acids supplied by these food acidulants may have an influence on zinc bioaccessibility from the food grains. Thus, the influence of these acidulants on zinc bioaccessibility from representative food grains has also been assessed in this investigation.

An *in vitro* procedure involving equilibrium dialysis during simulated gastrointestinal digestion was adopted throughout this investigation in all the above zinc and iron bioavailability determination studies. This method essentially measures the fraction of the total mineral in the food or diet that is available for uptake by the intestinal brush border cell membranes, in other words, the ‘bioaccessibility’ of the mineral. In view of the recent redefining of the terms related to bioavailability, we prefer to use the term ‘bioaccessibility’ when referring to the results obtained with this *in vitro* procedure.

Thus, the present investigation addressed the following objectives:

1. Determination of bioaccessibility of zinc and iron from staple cereals and pulses commonly consumed in India; inference of a possible correlation between mineral bioaccessibility and the inherent contents of phytate, calcium, tannin and dietary fibre; validation of the influence of phytate on zinc bioaccessibility by modulation of the phytate content of the grain.

2. Study of the influence of domestic cooking on zinc and iron bioaccessibility from these food grains.

3. Studies on the possible interaction of exogenous iron and calcium with zinc in relation to the bioaccessibility of the latter.

4. Evaluation of the influence of germination and fermentation of food grains on zinc and iron bioaccessibility.

5. Determination of the effect of food acidulants on zinc and iron bioaccessibility from food grains; zinc and iron bioaccessibility from cereal- based whole meals consumed in India.

Photograph of cereals studied for zinc and iron bioaccessibility

Photograph of pulses studied for zinc and iron bioaccessibility

CHAPTER – II

BIOACCESSIBILITY OF ZINC AND IRON FROM CEREALS AND PULSES CONSUMED IN INDIA

Bioaccessibility of Zinc and Iron from Cereals and Pulses Consumed in India

INTRODUCTION

Zinc is the fourth important micronutrient after vitamin A, iron and iodine, which is now receiving increasing global attention. Deficiency of this element, although not completely assessed, is believed to be as widespread as that of iron and is a cause for concern especially in the developing countries (Prasad, 2003). Although the cause of suboptimal zinc status in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are likely the most common causative factors. Although animal foods are rich sources of zinc in our diet, this micronutrient has to be derived mainly through food grains by a majority of the population in developing countries. Staple foods in developing countries include cereals and legumes which are the main sources of zinc for a majority of the population, but even if net zinc intake appears adequate, compromised zinc status is common (Gibson *et al*, 1998). In general, the average zinc intakes of vegetarians tend to be lower than those of their omnivorous counterparts (Gibson 1994).

Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of hemoglobin, myoglobin and cytochromes. Despite large scale intervention programmes, iron-deficiency anaemia remains the most widely prevalent nutritional problem in the world. Although many factors are responsible for the onset of iron deficiency, the most likely cause of this nutritional problem in developing countries is the poor bioavailability of dietary iron.

Bioavailability of micronutrients, particularly zinc and iron is low from plant foods (Gibson, 1994; Sandberg, 2002). Bioavailability of iron is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption. Among inhibitors, phytic acid, tannins, dietary fibre and calcium are the most potent, while organic acids are known to promote iron absorption (Gibson, 1994; Sandberg, 2002). Although not exhaustively evidenced, it is possible that the bioavailability of zinc from food grains is similarly influenced by such diverse factors coexistent in them. Cereals and pulses are known to contain high concentrations of one or more of the above inhibitors of iron absorption.

Food processing by heat generally alters the bioavailability of nutrients - both macro and micro. The digestibility and hence absorption of micronutrients such as iron is believed to be improved upon heat processing; with the resultant softening of the food matrix, protein-bound iron is released, thus facilitating its absorption (Lombardi-Boccia *et al*, 1995). In addition, heat-processing of food is also likely to alter the inherent factors that inhibit mineral absorption, such as phytate and dietary fibre, especially the insoluble fraction.

Information on the bioavailability of zinc from the food grains commonly consumed in India is limited. Such information would be useful for computing the Recommended Dietary Allowances (RDA), as well as for evolving a dietary strategy to improve micronutrient intake. In this investigation, several food grains commonly consumed in India were screened for their zinc content and its bioaccessibility. Incidentally, the iron content and its bioaccessibility from these food grains were also determined in order to make a comparison. Various factors inherent in these grains, which may have an influence on the bioaccessibility values of zinc and iron, namely, phytic acid, tannin, and calcium and dietary fibre were also quantitated. The bioaccessibility values of zinc and iron of the food grains studied have been correlated with concentrations of the above inherent factors.

In the absence of any information on the influence of heat processing of food grains on zinc bioavailability, it would be relevant to examine the same. The influence of domestic heat processing on the bioaccessibility of these minerals from the food grains has also been studied.

Supplementary studies were also conducted on selected grains, wherein the effect of removal of inherent phytate from the grains (rich in it), and addition of exogenous phytate to the grains (deficient in it), on zinc and iron bioaccessibility were examined.

MATERIALS AND METHODS

Materials:

Cereals - rice (*Oryza sativa*), finger millet (*Eleusine coracana*), sorghum (*Sorghum vulgare)*, wheat (*Triticum aestivum*) and maize (*Zea* mays), and pulses - chickpea (*Cicer arietinum*) - whole and decorticated, *g*reen gram (*Phaseolus aureus*) – whole and decorticated, decorticated black gram (*Phaseolus mungo*), decorticated red gram (*Cajanus cajan*), cowpea (*Vigna catjang*), and French bean (dry; *Phaseolus vulgaris*) were procured locally, cleaned and used here. Pepsin, pancreatin and bile extract, all of porcine origin, phytase (from *Aspergillus ficuum)*, and phytic acid (sodium salt) were from Sigma Chemical Co., USA. All other chemicals used here were of analytical grade. Triple distilled water was employed during the entire study. Acid-washed glassware was used throughout the study.

Total zinc, iron and calcium:

Grain samples were ground finely and ashed in a muffle furnace (Sardar Furnace) at 550^{o}C for 10 h and the ash was dissolved in conc. HCl. Zinc and iron content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). In the case of calcium, lanthanum chloride was added to the mineral solution to avoid interference from phosphate. Calibration of measurements was performed using commercial standards. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron.

Bioaccessibility of zinc and iron:

Bioaccessibility of zinc and iron from various food grain samples was determined by an *in vitro* method described by Luten *et al* (1996), involving simulated gastrointestinal digestion with suitable modifications. All food grain samples were finely ground in a stainless steel blender. The ground samples were subjected to simulated gastric digestion by incubation with pepsin (pH 2.0) at 37°C for 2 h. Titratable acidity was measured in an aliquot of the gastric digest, by adjusting the pH to 7.5 with 0.2 M sodium hydroxide in the presence of pancreatin-bile extract mixture (1 L 0.1M sodium bicarbonate containing 4 g pancreatin + 25 g bile extract). The titratable acidity was defined as the amount of 0.2M sodium hydroxide required to attain a pH of 7.5.

To simulate intestinal digestion, segments of dialysis tubing (Molecular mass cut off: 10 kDa) containing 25 ml sodium bicarbonate solution, being equivalent in moles to the sodium hydroxide needed to neutralize the gastric digest (titratable acidity) determined as above were placed in Erlenmeyer flasks containing the gastric digest and incubated at 37°C with shaking for 30 min or longer until the pH of the digest reached 5.0. Five ml of the pancreatin-bile extract mixture was then added and incubation was continued for 2 h or longer until the pH of the digest reached 7.0. At the end of simulated gastro-intestinal digestion, zinc and iron present in the dialyzate were analyzed by atomic absorption spectrometry. The dialyzable portion of the total mineral present in the sample (expressed as percent) represented the bioaccessible mineral.

Bioaccessibility (%) was calculated as follows: bioaccessibility (%) = 100 x Y/Z, where, Y is the element content of the bioaccessible fraction (mg mineral element /100 g grain), and Z is the total zinc or iron content (mg mineral element /100 g grain).

Phytate, tannin and dietary fibre:

Phytate in food grains was determined as phytin-phosphorus by the method of Thompson and Erdman (1982). Phytic acid values were computed by multiplying phytin phosphorus value by 3.55. Dietary fibre (soluble and insoluble) was estimated by enzymatic - gravimetric method as described by Asp *et al* (1983). Tannin was estimated by the modified vanillin assay of Price *et al*. (1978), using catechin as the standard.

Heat processing of food grains:

To examine the influence of heat processing on zinc and iron bioaccessibility from the food grains, two methods of heat processing - pressure cooking and microwave cooking were employed. Ten grams of the food grains were pressure-cooked in 30 ml of triple distilled water for 10 min (15 psi). In the case of French bean and chickpea whole the grains were soaked in triple distilled water overnight and then pressure cooked as above. For microwave cooking, 10 g of the food grains were cooked in 150 ml of triple distilled water at 360 Watts for 30 min in the case of whole grains (pre-soaked overnight) and 20 min for cereals and decorticated pulses (pre-soaked for 4 h). The cooked samples were homogenized in a stainless steel Omni-mixer (Sorvall) and used for the determination of mineral bioaccessibility as described above.

Statistical analysis:

All determinations were made in five replicates and the average values are reported. Statistical analysis of analytical data was done according to Snedecor and Cochran (1977). The data were also analyzed statistically by multiple regression tests, to infer the extent of modulation of zinc and iron bioaccessibility by inherent phytic acid, tannin, calcium and dietary fibre, and by Pearson moment correlation to infer the relationship between molar ratios of particular factors using the statistical software - SPSS programme.

Bioaccessibility of zinc and iron from selected food grains after partial removal of phytate:

Ground samples of decorticated green gram and black gram were pre-treated with commercial phytase to bring about hydrolysis of inherent phytate. The enzyme was added at a level of 2.2 U/ g sample suspended in 2M glycine-HCl buffer, pH 2.5, and incubated for 60 min at 37°C. At the end of phytase reaction, phytate content and bioaccessibility of zinc and iron from the above samples were determined as described above.

Influence of exogenous phytate on the bioaccessibility of zinc and iron from selected food grains:

The food grains studied for this purpose were rice, decorticated chickpea, French beans and cowpea. The exogenous phytic acid was added to the ground grains (10 g) at four different levels – equal to, two, three, and four times the inherent phytate content of the grains. Bioaccessibility of zinc and iron from these samples was determined as described above.

RESULTS AND DISCUSSION

The bioavailability of an inorganic nutrient is the portion of the total present in the food, meal or diet that is consumed. The physico-chemical form of metal ions has an important influence on their absorption from the intestines. Foods contain a large number of ligands for metal ions, such as proteins, peptides, amino acids, carbohydrates, lipids and inorganic ions (Watzke, 1998). These components in the diet form soluble and insoluble complexes with minerals and trace elements under gastro-intestinal conditions affecting their bioavailability.

Zinc and iron content in cereals and pulses:

Zinc and iron contents of the food grains studied are presented in Tables-1 and 2. The total zinc content of cereals ranged from 1.08 mg / 100 g in rice to 2.24 mg/ 100 g in sorghum, while that of pulses was between 2.03 mg/ 100 g (whole chickpea) and 2.68 mg / 100 g (decorticated chickpea). Thus, the inherent zinc concentration in cereals was lower, nearly half of that in pulses except in the case of sorghum, whose zinc concentration was comparable to that of pulses. Thus, all the pulses examined here, with a uniformly higher amount of zinc compared to cereals are a better source of this micronutrient. The iron content of cereals ranged from 1.32 mg% in rice to 6.51 mg% in sorghum, while that of pulses ranged from 3.85 mg% in decorticated green gram to 6.46 mg% in black gram. The iron concentration of rice and wheat was about 30% higher than that of zinc, while in finger millet and maize it was double and almost 3-times the zinc content in sorghum. In all the pulses examined, iron concentration was higher than the zinc concentration. Whole chickpea, red gram, black gram, and French bean had an iron content that was more than 2-times the zinc concentration.

Table- 1 Zinc and iron bioaccessibility from cereals

Food grain	Zinc Content (mg /100g)	Iron Content (mg/100g)	Zn / Fe Molar Ratio	Zinc Bioaccessibility (%)	Iron Bioaccessibility (%)
Rice	1.08	1.32	0.70	21.4	8.05
Wheat	1.62	3.89	0.36	8.93	5.06
Finger millet	1.73	2.13	0.70	8.31	6.61
Sorghum	2.24	6.51	0.29	5.51	4.13
Maize	1.48	3.21	0.39	7.82	7.83

Values are average of 5 replicates; the individual deviations from the mean value were less than 10%.

Table- 2 Zinc and iron bioaccessibility from pulses

Food grain	Zinc Content (mg /100g)	Iron Content (mg/100g)	Zn / Fe Molar Ratio	Zinc Bioaccessibility (%)	Iron Bioaccessibility (%)
Chickpea					
Whole	2.03	4.95	0.35	44.9	6.89
Decorticated	2.68	5.05	0.46	56.5	4.82
Green gram					
Whole	2.40	4.55	0.46	27.0	2.25
Decorticated	2.19	3.85	0.49	40.8	7.49
Red gram	2.35	4.93	0.42	45.7	3.06
Black gram	2.30	6.46	0.31	33.4	2.76
Cow pea	2.57	4.79	0.45	53.0	1.77
French bean	2.18	5.94	0.32	52.5	10.2

Values are average of 5 replicates; the individual deviations from the mean value were less than 10%.

Bioaccessibility of zinc and iron from raw cereals and pulses:

The bioaccessibility values of zinc present in various cereals and pulses as determined by the *in vitro* digestibility procedure are also presented in Tables-1 and 2. Pulses in general had higher amounts of bioaccessible zinc, which ranged from 27 to 56.5% of the element present, compared to cereals. Bioaccessibility of zinc from cereals ranged from 5.5 to 21.4% of the element present in them. Among the cereals, bioaccessibility of zinc was highest in rice (21.4%) and least in sorghum (5.51 %), while among pulses the highest was seen in decorticated chickpea (56.5%) and least in decorticated green gram (27.0%). Thus, bioaccessibility of zinc from pulses was several-fold higher than that from cereals. Among the cereals, the lowest availability of zinc from sorghum although it had the highest concentration of this element (2.24 mg%) and the highest availability of this mineral in the case of rice with the lowest concentration of zinc (1.08 mg%) indicated that zinc bioaccessibility is not necessarily dependent on its concentration in the food grain. Bioaccessibility of iron was almost similar from the five cereals examined and ranged from 4.13% in sorghum to 8.05% in rice. Bioaccessibility of iron from the examined pulses ranged from 1.77% in cowpea to 10.2 in French bean. Unlike that of zinc bioaccessibility, there was no striking difference between cereals and pulses with regard to iron bioaccessibility. Our bioaccessible iron percentages in whole and decorticated chickpea (6.89, and 4.82, respectively) was less than that reported by Chitra *et al* (1997) (25%), who measured only soluble ionic iron rather than total soluble iron. The bioaccessibilities of zinc from chickpea obtained in this study (44.9 and 56.5% for whole and decorticated forms, respectively) are comparable to the 54.7% obtained by Jood and Kapoor (1997).

Among pulses, whole grains of chickpea and green gram had lower availability of zinc compared to their decorticated counterparts, indicating that the seed coat might consist of components that inhibit the bioavailability of minerals.In general, the bioaccessibility of zinc from all the food grains studied was higher than that of iron. This difference was more prominent in the case of pulses. Zn: Fe molar ratio in cereals ranged from 0.29 to 0.7 and from 0.32 to 0.49 in pulses (Tables-1 and 2). Zn: Fe molar ratio was < 1 in all the food grains examined. Thus, despite the lower molar concentrations of zinc relative to that of iron, zinc bioaccessibility was always higher than that of iron, in all the food grains examined.

In the case of iron, which is present in both divalent and trivalent forms, the former is known to be readily absorbed. The higher bioaccessibility of zinc from food grains relative to iron could probably be due to the existence of zinc only in one valent state, namely the divalent form. The exceptionally higher bioaccessibility of zinc from rice could be attributed to the low levels of inhibitors of its absorption through interference with solubilization of the minerals, such as phytic acid, calcium, tannin and dietary fiber present in this grain. The amount and / or quality of protein may influence trace element bioaccessibility (O'Dell, 1984). High protein diets, especially those based on animal protein, are reported to enhance the bioavailability of trace minerals, probably by formation of soluble amino acid complexes, which facilitate absorption of the former (Snedeker & Greger, 1983). This may probably explain the higher bioaccessibility of zinc from pulses than from cereals, the former being richer sources of proteins.

Minerals that are similar in chemical configuration are likely to compete with each other at the site of absorption, thus coming in the way of their bioavailability (Gibson, 1994). It is not known if such factors would similarly influence zinc bioavailability from these food grains. When the zinc bioaccessibility from cereals in this study was viewed in relation to the inherent Zn: Fe molar ratio, although there was a slight trend towards increased *in vitro* availability of zinc with increased Zn: Fe molar ratio of the grains (Fig.1), such a trend was however not discernible in the case of pulses (Fig.2). This could be due to the presence of other dominating factors such as phytate, which overshadow the effect of Zn: Fe molar ratio alone in pulses. It should be inferred that iron probably does not affect zinc absorption at the molar ratios inherent in the food grains. Iron in pharmacological supplements however, has been reported to decrease zinc absorption in humans, especially when given in a water solution rather than with the diet (Troost *et al*, 2003). When iron bioaccessibility from food grains was viewed in relation to the inherent Zn: Fe molar ratio (Figs.3 and 4), it was found that iron dialysability was independent of zinc concentration. Thus, the Zn: Fe molar ratio is probably not a major determinant of the *in vitro* availability of these minerals at the concentrations they are normally present in the food grains.

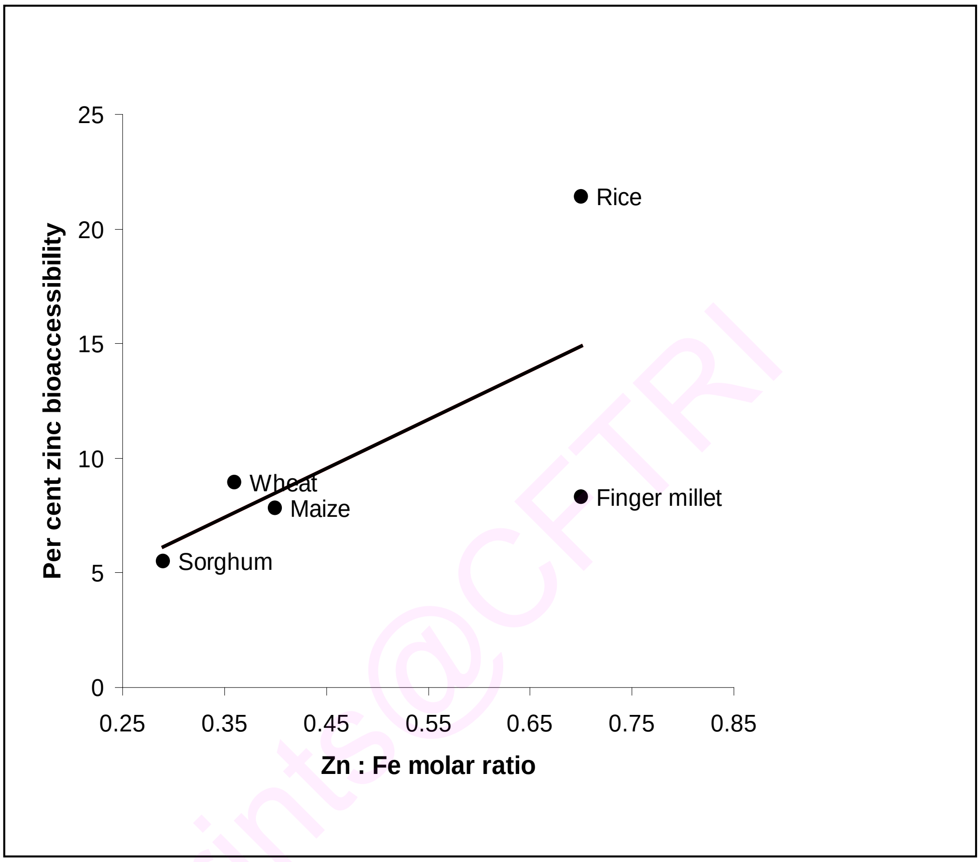


Fig.1 Zinc bioaccessibility from cereals in relation to inherent Zn: Fe molar ratio

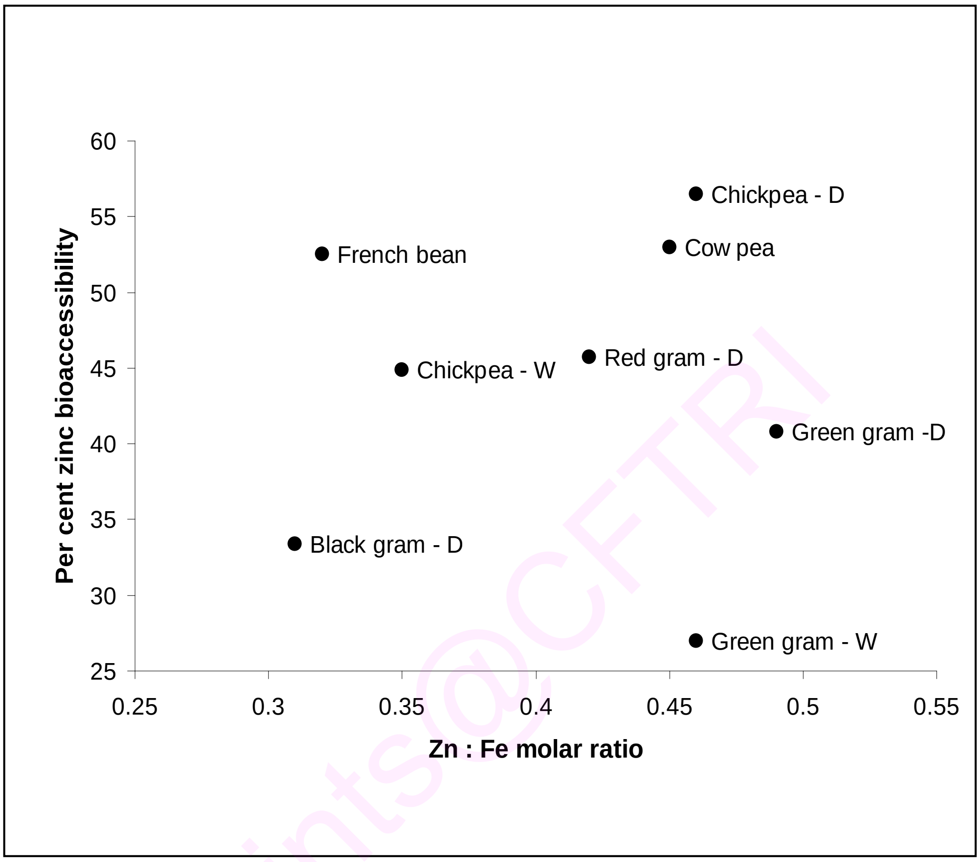


Fig.2 Zinc bioaccessibility from pulses in relation to inherent Zn: Fe molar ratio

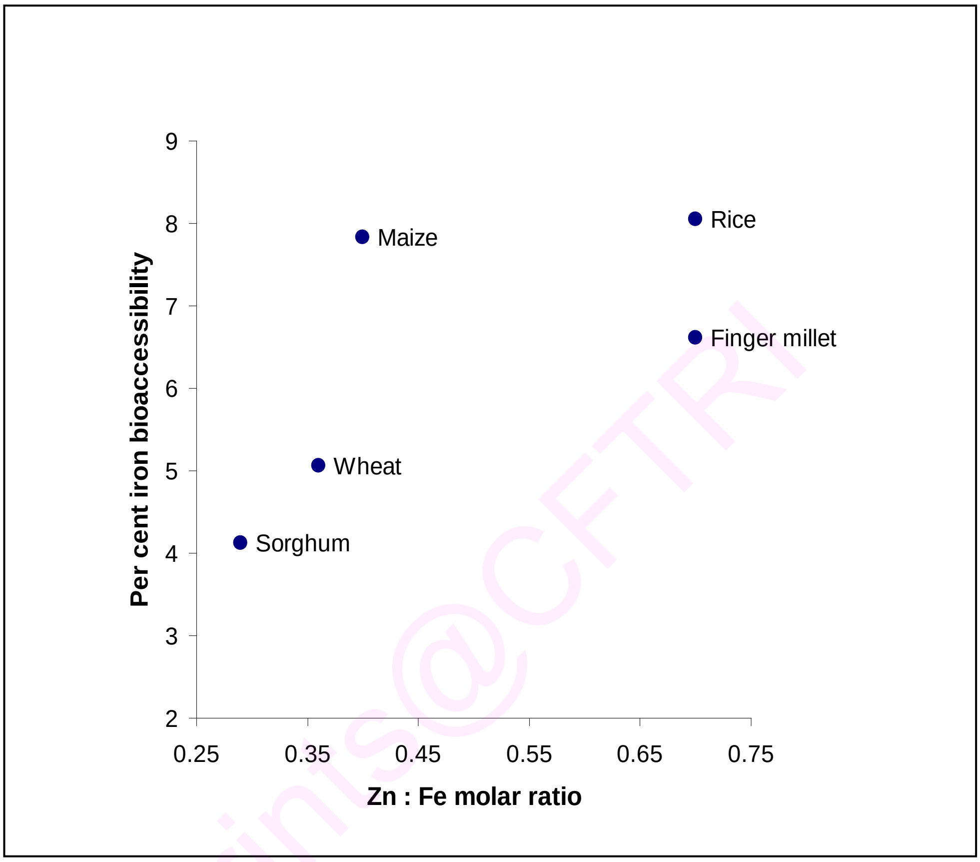


Fig.3 Iron bioaccessibility from cereals in relation to inherent Zn: Fe molar ratio

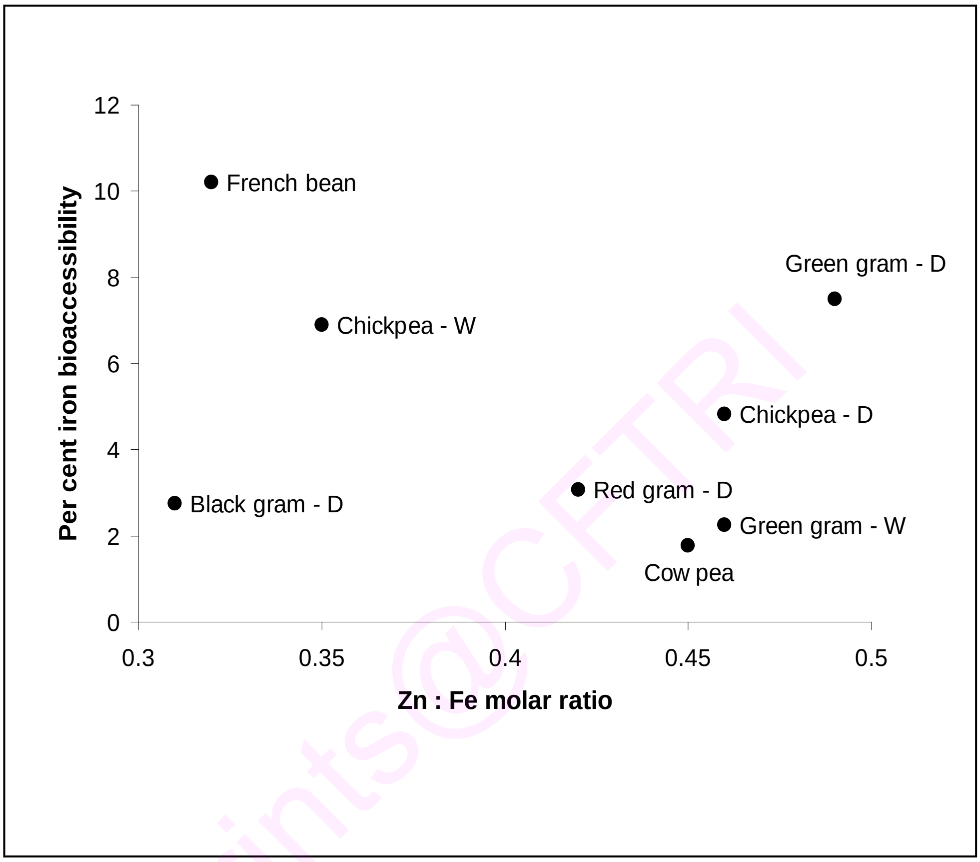


Fig.4 Iron bioaccessibility from pulses in relation to inherent Zn: Fe molar ratio

W: whole grain D: decorticated grain

Concentrations of phytic acid, calcium, tannin and dietary fibre in food grains:

Thus, we observed that bioaccessibility of zinc from pulses (27- 56%) is generally higher than that from cereals (5.5 – 21.4%), despite similar amounts of zinc present in both pulses and cereals. On the other hand, bioaccessibility of iron was almost similar from these cereals and pulses, in spite of differences in their iron content. Such a difference in the mineral bioaccessibility values could probably be attributed to various inherent factors associated with the grains. In this context, the concentrations of various factors such as phytic acid, tannin, calcium and dietary fibre inherent in these grains, which are likely to influence the bioaccessibility values of zinc and iron, were determined. The bioaccessibility values of zinc and iron of the food grains studied have been correlated with concentrations of the above inherent factors.

Concentrations of phytic acid, tannin and calcium in various food grains examined for zinc bioaccessibility are presented in Tables-3 and 4. It was observed that phytic acid content of the cereals examined ranged from 160 mg% in rice to 612 mg% in wheat, while that in pulses was from 263 mg% in whole chickpea to 630 mg% in decorticated green gram. Apart from decorticated green gram, whole green gram (553 mg%) and decorticated black gram (539 mg%) had relatively higher amounts of phytic acid, among pulses. In the case of chickpea and green gram, the decorticated grains had a higher phytic acid concentration than their whole grain counterparts. This is because phytic acid is mainly located in the cotyledon, with the hull containing less than 0.1% of the seed phytate (Carnovale *et al*, 1988).

The calcium content of the food grains tested ranged from 7.2 mg % in maize, to 325 mg% in finger millet. In general, calcium content of cereals was lower than that of pulses, except in finger millet. Among pulses, French beans and whole chickpea had especially high amounts of calcium (158 and 141 mg%, respectively).

Among the food grains tested, finger millet had the highest amount of tannin (2.12 g%) and similar high amounts were present in French beans (3.08 g%) and cowpea (2.21 g%). Rice had the least amount of tannin (5 mg%), followed by maize (19 mg%).

Table-3 Phytic acid , calcium and tannin contents of cereals

Food grain	Phytic acid	Calcium	(Phytate) X (Calcium) / (Zinc) Molar ratio	Tannin
Rice	159.80	7.26	14.66	5.00
Wheat	612.40	36.90	37.46	211.70
Finger millet	417.10	325.00	23.90	2116.70
Sorghum	294.70	14.30	13.04	68.30
Maize	414.30	7.19	27.74	19.20

Values (mg/100 g) are average of 5 independent determinations

Table-4 Phytic acid , calcium and tannin contents of pulses

Food Grain	Phytic Acid	Calcium	(Phytate) X (Calcium) / (Zinc) Molar ratio	Tannin
Chickpea				
Whole	263.4	141.1	12.86	146.7
Decorticated	324.1	54.1	11.98	58.3
Green gram				
Whole	553.1	90.3	22.84	575.0
Decorticated	630.1	49.2	28.50	43.30
Red gram	387.7	48.0	16.35	65.00
Black gram	538.5	67.9	23.20	55.80
Cowpea	378.8	87.9	14.61	2208.3
French beans	369.9	158.2	16.81	3075.0

Values (mg/100 g) are average of 5 independent determinations

Dietary fibre content of various cereals and pulses examined is given in Tables-5 and 6. Total dietary fibre content was lowest in the case of rice, viz., 3.65%. Whole grains had higher dietary fibre than their respective decorticated forms in the case of chickpea (27.3 vs. 8.76%) and green gram (22.3 vs. 8.37%).The amounts of fibre in these whole legumes were similar to those of other whole legumes - French bean and cowpea (25.8 and 22.5%, respectively), studied here. This higher fibre in the whole legumes is contributed essentially by the insoluble fraction present in the husk portion. The insoluble fibre content of decorticated pulses and of cereals except rice was similar. The soluble dietary fibre fraction was especially higher in the case of French beans (4.55%).

Correlation between bioaccessibility of zinc and iron and various inherent factors:

Influence of phytic acid:

When zinc bioaccessibility values of the food grains tested were viewed with respect to the phytate content of the grain (Figs.5 and 6), a negative correlation between dialysability of zinc and phytate content was generally evident in both cereals and pulses. This negative correlation observed in the scatter diagram was corroborated by multiple regression analysis. The multiple regression analysis carried out to explain the influence of phytic acid on zinc bioaccessibility (Table-7) indicated that this negative effect of phytic acid was highly significant (1%) in the case of pulses, while in cereals, the same was not statistically significant.

However, when the negative influence of phytic acid on zinc bioaccessibility was viewed in terms of phytate: zinc molar ratio present in the grain (Figs. 5 and 6) rather than the absolute amounts of phytate independent of zinc concentration, the following inference can be drawn: In the case of cereals, increase in the molar ratio of phytate: zinc from 14.7 in rice to 23.9 in finger millet was correlated with a drastic decrease in zinc bioaccessibility, while further increase in this ratio even up to 37.5 in wheat had no corresponding negative effect. Although sorghum had a phytate: zinc molar ratio of 13, it displayed a much lower zinc bioaccessibility than that of rice with a phytate: zinc molar ratio of 14.7, and was comparable to the other tested cereals with higher phytate: zinc molar ratios. This could be attributable to the presence of factors other than phytic acid such as tannin acting in synergy. A similar trend was observed in pulses up to a phytate: zinc molar ratio of 22.8 (in whole green gram), beyond which the negative trend was not seen. However, statistical analysis of this data revealed that this negative correlation of phytate: zinc molar ratio with zinc bioacessibility was not significant.

Table- 5 Dietary fibre content of cereals

Food grain	Total	Insoluble	Soluble
Rice	3.65	2.80	0.85
Wheat	10.9	8.30	2.60
Finger millet	13.1	12.0	1.10
Sorghum	10.2	9.40	0.80
Maize	11.5	8.83	2.70

Values are expressed as g / 100g and are average of five independent determinations; the individual deviations from the mean value were less than 10%.

Table- 6 Dietary fibre content of pulses

Food grain	**Total**	**Insoluble**	**Soluble**
Chickpea Whole	27.3	24.4	2.97
Decorticated	8.76	7.90	0.86
Green gram Whole	22.3	21.0	1.38
Decorticated	8.37	6.90	1.47
Red gram	12.6	10.2	2.50
Black gram	16.5	15.2	1.30
Cowpea	22.5	20.7	1.75
French bean	25.8	21.3	4.55

Values are expressed as g / 100g and are average of five independent determinations; the individual deviations from the mean value were less than 10%.

Table- 7 Estimated regression function depicting the influence of various inherent factors in relation to zinc bioaccessibility

Inherent factors	Regression Co-efficient	
	Cereals	Pulses
Constant	27.949 (1.473)	78.29 (16.46)
Zinc	-1.631* (0.532)	-0.766 (2.468)
Calcium	-0.040 (0.035)	0.083 (0.117)
Phytic acid	-0.005 (0.005)	-0.047** (0.017)
Soluble dietary fibre	-2.326* (0.885)	0.455 (3.068)
Insoluble dietary fibre	-0.878 (0.494)	-1.249* (0.569)
Tannin	0.007 (0.005)	0.006 (0.003)
	R^2 = 0.955**	0.466 *

The Values in the parenthesis indicate standard error of the co-efficient
∗ Indicates significance at 5% level
∗∗ Indicate significance at 1% level

In the case of iron, phytic acid content of the cereal grains produced a proportionate negative influence on its bioaccessibility (Figs.7 and 8), with the exception of sorghum. This negative effect of inherent phytate on iron bioaccessibility from cereals was statistically significant (5%), as revealed by the multiple regression analysis. However, a similar negative influence of phytate on iron bioaccessibility from pulses was not evident (Table 8). When the influence of phytate on iron bioaccessibility was viewed in terms of phytate: iron molar ratio inherent in the grain, such a negative influence was not discernible. In the case of pulses in our study, the inverse relationship of iron dialysability with phytate concentration was generally evident, the exception being in the case of French bean and decorticated green gram (Fig.8). Decorticated green gram had a higher iron bioaccessibility despite higher phytate content compared with its whole counterpart.

Phytic acid (myoinositol hexaphosphate) is the principal storage form of phosphorous in cereals, legumes and oil seeds. It is the most potent inhibitor of zinc absorption, but also has a negative impact on non-heme iron absorption especially in foods of plant origin (Davies *et al*, 1977; Sandberg & Svanberg, 1991), forming insoluble chelates with iron and zinc in the intestine, that are unavailable for absorption (Wise, 1983; Sandstrom, 1989). The inhibitory effect of phytate on the absorption of zinc and to a lesser extent for non-heme iron has been reported to be dose-dependent (Hallberg *et al*, 1989). Several investigators have tested the phytate to zinc molar ratio to predict the bioavailability of zinc in phytate-containing diets using animal models. Such experiments showed that phytate: zinc molar ratios < 12 had little effect on the bioavailability of zinc in rats. The inhibitory effect of phytate content of foods on zinc bioavailability has been reported by Oberleas and Harland (1981), and Turnland *et al* (1984), and molar ratios of phytate to zinc of more than 12 have been implicated in the interference with zinc bioavailability in humans to compromise with zinc bioavailability in humans. Higher values such as 15 (Sandberg *et al*, 1986) and even 20 (Ferguson *et al*, 1989) of this critical ratio have been associated with clinical zinc deficiency in humans. In our study, although zinc bioavailability value of the food grain did not have a correlation when viewed in relation to the phytate: zinc molar ratios, nevertheless, a significant negative correlation were inferred when viewed in terms of absolute concentration of the inherent phytate, especially in pulses.

Table- 8 Estimated Regression function depicting the influence of various inherent factors on to iron bioaccessibility

Inherent factors	Regression Co-efficient	
	Cereals	Pulses
Constant	8.287 (1.015)	-0.095 (5.869)
Zinc	-0.776 (0.858)	0.651 (1.861)
Calcium	0.009 (0.017)	0.0918* (0.022)
Phytic acid	-0.006* (0.002)	0.0037 (0.003)
Soluble dietary fibre	1.43* (0.481)	0.470 (0.618)
Insoluble dietary fibre	-0.104 (0.175)	-0.427* (0.104)
Tannin	-0.0004 (0.003)	-0.0006 (0.001)
	R^2 = 0.792*	0.000**

The Values in the parenthesis indicate standard error of the co-efficient
∗ Indicates significance at 5% level
∗∗ Indicate significance at 1% level

Only the hexa- and penta- phosphate esters of inositol appear to significantly inhibit the bioavailability of iron and zinc (Brune *et al*, 1992; Lonnerdal *et al*, 1989). Inositol esters with fewer phosphate groups probably have a limited negative effect, or even have a positive effect. It becomes evident, therefore, that 'total phytate' of a meal or a diet is a crude measure when evaluating zinc bioavailability; methods that specifically quantify the various forms of inositol phosphates are needed when assessing the effect of phytate on zinc absorption. Using an *in vitro* Caco-2 cell model, Glahn *et al* (2002) did not detect a significant relationship between phytate as measured by inositol penta and hexa phosphates and iron bioavailability from rice. In an earlier study however, Glahn *et al* (2002a) showed that an iron – phytic acid molar ratio of 1:10 maximally inhibited iron bioavailability in Caco-2 cells, & that further increases in phytate produced no additional inhibition. The method employed in our study did not differentiate the various phosphates of inositol.

Influence of calcium:

The negative impact of calcium content in food on the availability of iron is well recognized, and this may probably also be true of zinc bioaccessibility (Davies *et al*, 1985). When zinc bioaccessibility of the grains examined here was viewed in relation to calcium concentration (Figs. 9 and 10), a decreasing trend was evident in cereals with increase in calcium concentration, which was however not statistically significant, as indicated by the multiple regression analysis. It appears unlikely that the Ca levels commonly found in mixed human diets will promote a phytate-induced decrease in Zn availability (Forbes *et al*, 1984). Some lacto-ovo vegetarians, however, who consume diets, which are relatively high in both phytate & calcium, but low in zinc, may be at a risk for suboptimal zinc nutriture. Under such conditions, [phytate] x [calcium]/ [zinc] molar ratio may provide a more useful assessment of zinc bioavailability than phytate: zinc molar ratio alone (Davies *et al*, 1984). In view of the potentiation of the inhibitory effect of phytate by calcium, other authors also have advocated the need for viewing the effect of calcium in terms of the [phytate] x [calcium] / [zinc] molar ratio (Ferguson *et al*, 1989; Bindra *et al*, 1986).

Figs.9 and 10 also depicts zinc bioaccessibility of various food grains viewed in relation to calcium concentration in terms of molar ratio: [phytate] x [calcium] / [zinc]. Generally in both cereals (Fig.9) and pulses (Fig.10), as this ratio increased, there was a decrease in zinc bioaccessibility from the food grain.

However, this negative correlation was not statistically significant. Very limited data exist on the critical [phytate] x [calcium] / [zinc] molar ratio inhibiting the bioavailability of zinc in human diets; although, a ratio between 150 and 200 has been suggested from a retrospective calculation of the data from Cossack and Prasad (1983). None of the food grains studied by us had [phytate] x [calcium] / [zinc] molar ratios exceeding this critical value. In fact, this ratio in all the grains studied here was less than 65, except finger millet (190).

Such an inverse relationship of calcium was however not evident on iron bioaccessibility values when the same were viewed in relation to calcium inherent in the grain, as well as [phytate] x [calcium] / [iron] molar ratio (Figs.11 and 12). On the other hand, calcium seems to have a significant positive influence on iron bioaccessibility in the case of pulses. The positive influence of calcium on iron bioaccessibility could be due to its forming a ligand with phytate, thus sparing iron and allowing its free accessibility (Anderson, 2000). The effect of dietary phytate on the bioavailability of zinc depends on the amounts of calcium in the diet relative to phytate. Maximum precipitation of phytate and therefore maximum chelation of zinc in solution occurs at Ca: phytate ratios > 6: 1, the percentage chelated decreasing with decreasing molar ratios (Wise, 1983). The critical Ca: Phytate molar ratio for maximum dietary zinc precipitation for humans is unknown although it has been suggested that zinc absorption is improved at a Ca: phytate molar ratio of 4.7 compared with a ratio of 8.0 when phytate: Zn molar ratios were > 20 (Navert *et al*, 1985)

Influence of tannin:

When the zinc and iron bioaccessibility values from various food grains were viewed in relation to their tannin content, it appeared that tannin did not have any significant influence on zinc and iron bioaccessibility from cereals and pulses (Figs.13 and 14; Tables-7 and 8). Among the various inherent factors examined, tannin accounted for only 0.7 and 0.6% of influence in cereals and pulses, respectively (Table-7). Similarly for iron, tannin accounted for 0.04 and 0.06% of influence in cereals and pulses, respectively (Table-8).

The negative role of tannin on iron absorbability from grains is equivocal. Tannin is reported to be a potent inhibitor of iron absorption (Hamdaoui *et al*, 1995). Several studies suggested that tannins exert a marked inhibitory effect on Fe absorption (Gillooly *et al*, 1983; Brune *et al*, 1989) and further this effect has been observed to be dose related (Siegenberg *et al*, 1991; Tutanwiroon *et al*, 1991). On the other hand, Lombardi-Boccia *et al* (1995) did not find any improvement in iron dialysability from *Phaseolus vulgaris* beans after removal of the hulls, which contain most of the seed tannin. So also in our study, tannin at the levels present in the grains did not exert any statistically significant negative effect on the bioaccessibility of either of the minerals.

Influence of dietary fibre:

When the bioaccessibility of zinc from various cereals and pulses was viewed in relation to the dietary fibre content- total, insoluble and soluble (Figs.15 and 16), it was evident that in general, there was a negative influence of total dietary fibre on the zinc bioaccessibility in the case of cereals (Fig.15). Although this trend was not consistent in the case of pulses, majority of the grains conformed to it (Fig.16). When the individual dietary fibre fractions of the food grains were related to zinc bioaccessibility value from cereals (Fig.15), both soluble and insoluble fractions negatively influenced zinc bioaccessibility, the effect being statistically significant (5% level) only for the soluble fibre fraction (Table-7). In the case of pulses, the insoluble fraction had a significant (5% level) negative influence on zinc bioaccessibility (Table-7).

Similar to the effect on zinc bioaccessibility, insoluble dietary fibre negatively influenced iron bioaccessibility from cereals and pulses (Figs.17 and 18), the effect being statistically significant (5% level) in the latter (Table-8). On the other hand, soluble dietary fibre fraction had a positive influence on iron accessibility from cereals and pulses, as indicated by the multiple regression analysis (Table-8), the effect being significant (5% level) in the case of cereals (although the scatter diagram indicated to the contrary).

Thus, while both insoluble and soluble fractions of the dietary fibre in the food grains generally interfered with zinc bioaccessibility, the insoluble fraction alone had this effect on iron bioaccessibility. Soluble fraction, on the other hand, had, in fact, the opposite effect on iron bioaccessibility, more so in the case of cereals. It is speculated that the amount and type of dietary fibre matters in their influence on trace element bioavailability (Gibson, 1994). In general, the soluble fibre pectin, found predominantly in fruits, does not affect the absorption of zinc. By contrast, insoluble cereal and vegetable fibre (cellulose, hemicelluloses, and lignin) are said to inhibit the bioavailability of zinc (Schwartz *et al*, 1986). The effects of dietary fibre on the bioavailability of trace elements are speculated to be confounded by the presence of other minerals and proteins in the food, as well as by the presence of phytate or oxalic acid (Kelsay *et al*, 1988). Oxalic acid forms fiber-zinc-oxalate complexes that are less readily degraded in the digestive tract than are the corresponding mineral-fibre complexes alone. The effect exerted on iron availability by the components of fibre fractions has not yet been clearly ascertained, while some fibre components have been proven to bind iron *in vitro*, many studies indicate that they have little effect on iron absorption (Torre *et al*, 1991; Rossander *et al*, 1992). Dietary fibre also has the potential to bind zinc and hence decrease the amount available for intestinal absorption (James, 1980). However, the negative influence of dietary fibre on zinc absorption / bioavailability needs to be substantiated.

Relative effects of all inherent factors on zinc and iron bioaccessibility:

In the case of zinc bioaccessibility from cereals, the multiple regression analysis fitted to explain the collective influence of various inherent factors listed in Table-7 indicated that the inherent factors explained a 95.5% (R^2) variation in the accessibility of zinc due to variation in the included factors, which is highly significant (1%). The regression model fitted here is satisfactory in explaining the variation in zinc bioaccessibility. In the case of pulses, the factors included explained 46.6% variation, which was found to be significant at 5% level (Table-7).

Thus, the lower collective negative influence of the various inherent factors on zinc bioaccessibility from pulses is consistent with the higher zinc bioaccessibility value of these grains, relative to cereals.

In the case of iron bioaccessibility from cereals, the collective influence of the inherent factors examined was to an extent of 79.2% (R^2), which was significant at

5% level (Table-8). The extent of collective influence of the various inherent factors on iron bioaccessibility from pulses was essentially similar (R^2 = 75.3%), which is statistically highly significant at 1% level (Table-8). This explains the previously observed similar iron bioaccessibility values in both cereals and pulses.

Influence of reduction of phytate by treatment with phytase on mineral bioaccessibility:

Among the various factors, phytate seems to have the maximum negative influence on the bioaccessibility of zinc, especially from pulses. This led us to examine whether removal of phytate from pulses would beneficially influence their zinc bioaccessibility. Decorticated green gram and black gram, which had relatively higher phytate content, were pre-treated with fungal phytase (Sigma Chemical Co, USA), which resulted in a 36 and 24% reduction in phytate in green gram and black gram, respectively. Fig.19 presents the zinc and iron bioaccessibility values from phytase-treated legumes. Reduction in phytate brought about a marginal increase (16%) in the bioaccessibility of zinc from black gram, while it did not seem to have any influence on the same from green gram. On the other hand, iron bioacessibility improved significantly as a result of reduction in phytate content, the increase being to an extent of 221% for black gram, and 74.5% for green gram. Thus, partial removal of phytate from the food grains enhanced the bioaccessibility of minerals, especially of iron, supporting our inference of a negative correlation of inherent phytic acid with zinc and iron bioaccessibility.

Thus, removal of phytate from food grains will prove advantageous in terms of increase in the bioaccessibility of iron and zinc. There are several methods available to reduce the phytate content of various foods: leavening of bread (Navert *et al*, 1985), fermentation (Gibson *et al*, 1998), germination & milling (Gibson *et al*, 1998; Svanberg & Sandberg, 1988), treatment of foods with food grade phytase or the addition of phytase to the diet (Sandberg *et al*, 1996; Turk & Sandberg, 1992).

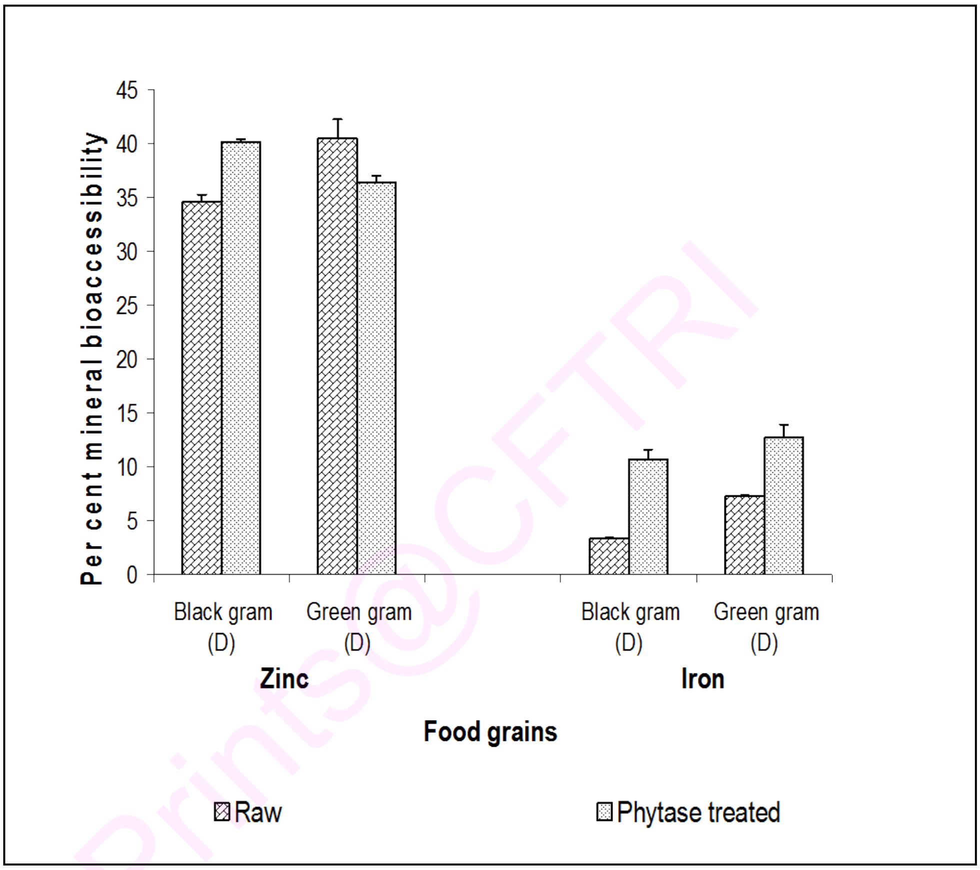


Fig.19 Zinc and iron bioaccessibility from phytase-treated grains

D: decorticated grain

Influence of addition of exogenous phytate on mineral bioaccessibility:

In a separate experiment, the effect of addition of exogenous phytic acid on the bioaccessibility of zinc and iron from food grains that were low in endogenous phytate was also examined. The food grains studied here were rice, decorticated chickpea, French bean and cowpea. The exogenous phytic acid was added at levels - equal to, twice, thrice and four times the endogenous level. Fig.20 presents the influence of exogenous phytate on zinc bioaccessibility from the four food grains examined.

Exogenous phytic acid showed only a marginal decreasing effect on zinc bioaccessibility from the food grains studied, the effect being significant only in a few instances.This effect, however, was not proportionate to the level added. The influence of exogenous phytic acid on iron bioaccessibility is presented in Fig.21. Since inherent phytic acid had a negative correlation with zinc or iron bioaccessibility, presence of additional amounts of phytic acid is expected to further decrease the mineral bioaccessibility values. However, contrary to this expectation, it was found that iron bioaccessibility values were profoundly enhanced from all the food grains tested. The extent of increase of iron bioaccessibility was as high as 2.8-fold in the case of rice, 3.8-fold in the case of French bean and 6.6-fold in the case of cowpea, at the highest level of addition of exogenous phytic acid. The increase in iron bioaccessibility was also almost proportional to the added phytic acid in these grains.

This unexpected trend in the influence of phytic acid exogenously added cannot be explained within the limitations of the experiment performed. It is suggested that while inositol hexa- and penta- phosphates are negative influencers of mineral bioaccessibility, inositol tetra- and tri- phosphates have been reported to enhance iron bioavailability (Sandberg *et al*, 1999). The latter two are the intermediates during phytic acid hydrolysis either by heat or enzymatic treatment. The observed promotive effect of exogenous phytic acid on iron bioaccessibility from the test food grains in the current study is probably attributable to the possible presence of tetra- and tri- inositol phosphates in the sample used for exogenous phytic acid addition, which needs to be verified.

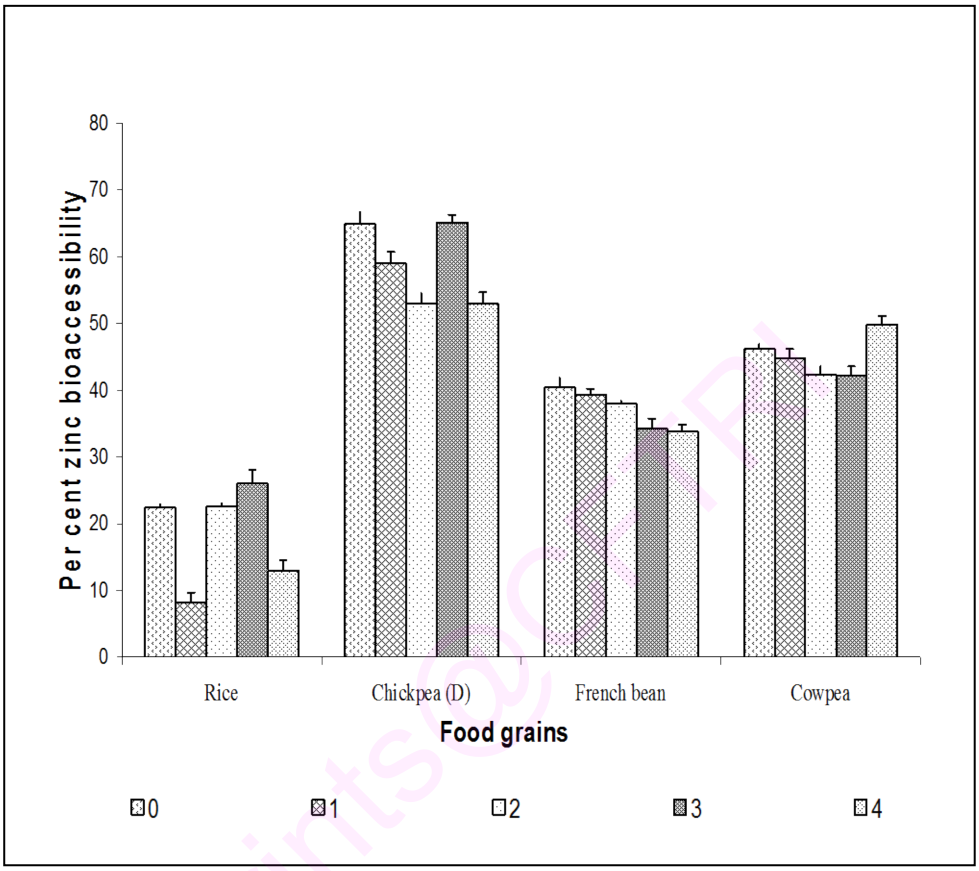


Fig.20 Zinc bioaccessibility from grains in presence of exogenous phytic acid

0: No addition
1: Native level + 1 X native level
2: Native level + 2 X native level
3: Native level + 3 X native level
4: Native level + 4 X native level

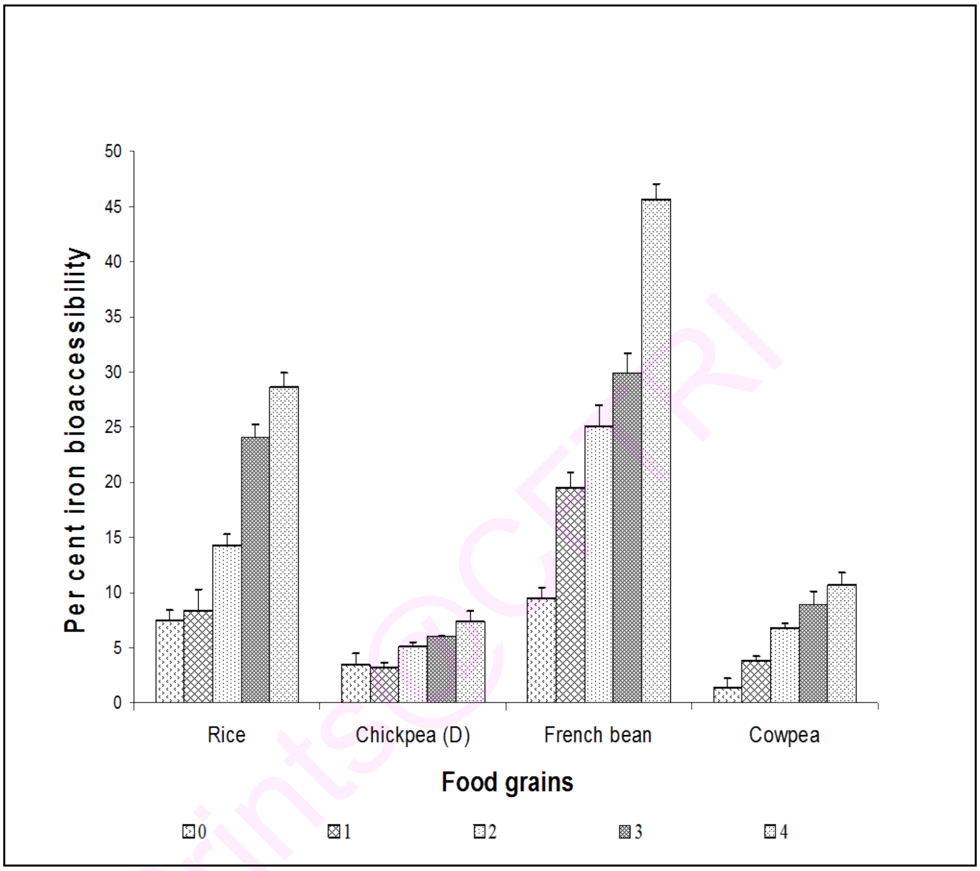


Fig.21 Iron bioaccessibility from grains in presence of exogenous phytic acid

0: No addition
1: Native level + 1 X native level
2: Native level + 2 X native level
3: Native level + 3 X native level
4: Native level + 4 X native level

Effect of heat processing on zinc and iron bioaccessibility:

All the food grains (both cereals and legumes) studied in this investigation are consumed in the cooked form, while a few of the legumes are also consumed in raw form after soaking and germination (as salads). Considering the bioaccessibility of zinc and iron from the cooked form of these grains, would therefore be more meaningful. Influence of heat processing on the zinc bioaccessibility from various cereals and pulses is presented in Figs.22 and 23. Heat treatment of the food grains by pressure-cooking generally decreased the bioaccessibility of zinc, especially in pulses. Among the cereals examined (Fig.22), pressure-cooking decreased the bioaccessibility of zinc by an extent of 63 and 57% in the case of finger millet and rice, respectively.

On the other hand, there was a significant increase (72%) in the bioaccessibility of zinc from sorghum as a result of pressure-cooking. Microwave cooking also brought about similar decrease in zinc bioaccessibility in the case of rice (39%) and maize (19%).The bioaccessibility of zinc from finger millet, on the other hand, increased by an extent of 23% upon microwave cooking. In the case of wheat, neither of the heat treatment procedures affected the bioaccessibility of zinc to any significant extent. Thus, all the pressure- cooked cereals had similar percent zinc bioaccessibility values, with the exception of finger millet, which had a much lower percent zinc bioaccessibility. There was generally a significant decrease in the bioaccessibility of zinc from all the pulses studied, as a result of heat treatment by pressure-cooking or microwave cooking (Fig.23). This decrease ranged from 11.4% in microwave-cooked chickpea (whole), to 63% in microwave cooked cowpea. Decrease in zinc bioaccessibility was 48% in pressure-cooked whole chickpea, 45 and 55% in heat processed whole green gram, 32 and 22% in heat processed decorticated green gram, 45% in microwave-cooked black gram. As in the case of raw grains, the cooked decorticated pulses had higher concentrations of bioaccessible zinc than their whole counterparts. In general, microwave cooking did not seem to have any advantage over pressure-cooking with respect to bioaccessibility of zinc.

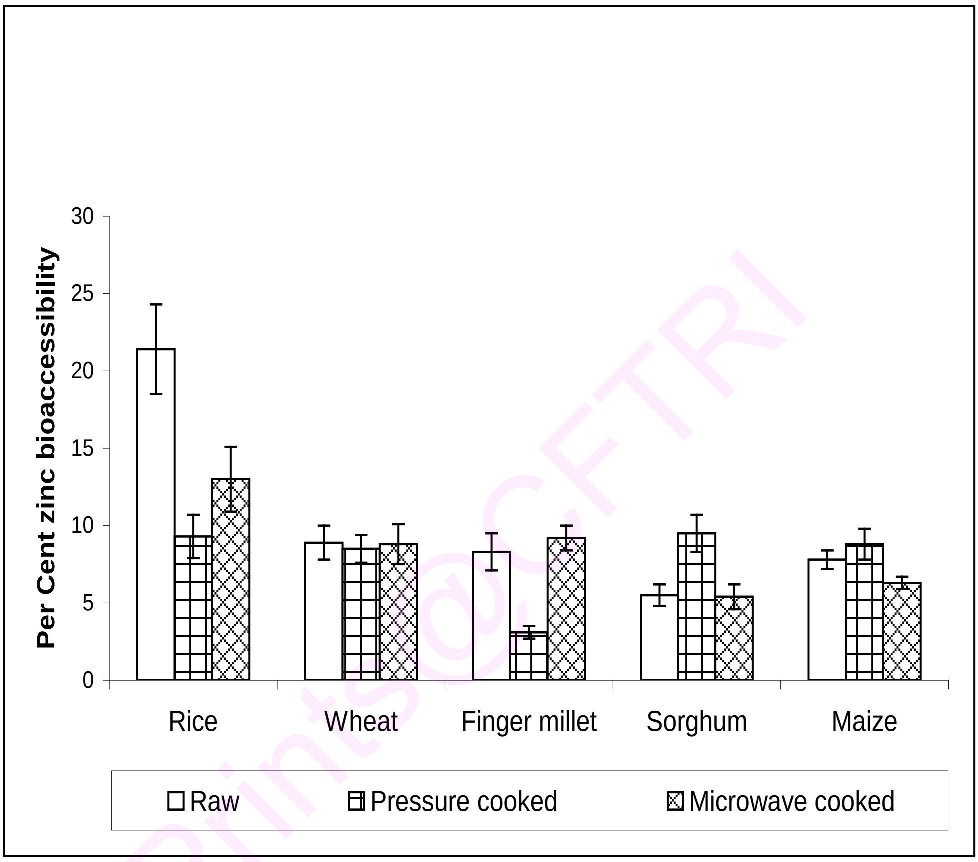


Fig.22 Influence of heat processing on zinc bioaccessibility from cereals

Values are mean ± SEM of 5 replicates.

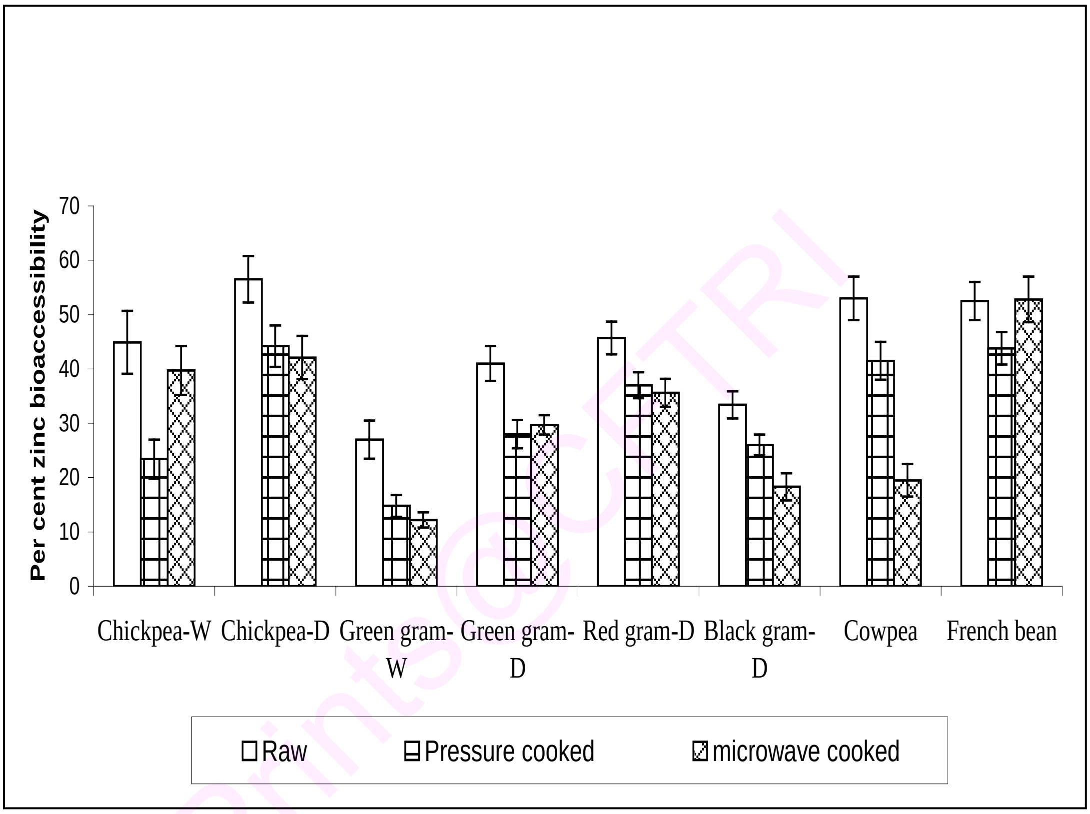


Fig.23 Influence of heat processing on zinc bioaccessibility from pulses

Values are mean ± SEM of 5 replicates.

W: Whole grain D: Decorticated grain

Kaur and Kawatra (2002) have reported a higher retention of zinc in rats fed diets based on dehusked, soaked and pressure-cooked rice bean. Soaking and dehusking of rice bean followed by pressure-cooking resulted in a greater percentage of soluble zinc. The same was observed in rice bean that was sprouted and pressure-cooked. This higher solubility was attributed to a decrease in antinutritional factors inherently present in this legume, as a result of soaking / sprouting. In the present study, consistently lower concentrations of bioaccessible zinc were observed in the heat-treated food grains. This trend was evident even in un-dehusked legumes, and those soaked overnight prior to heat treatment.Contrary to its effect on the bioaccessibility of zinc, heat treatment, either pressure-cooking or microwave cooking, generally increased the bioaccessibility of iron from all the food grains studied (Figs.24 and 25). The bioaccessibility of iron from pressure-cooked cereals was uniform and ranged from 7 to 9.5%, except in the case of rice (12%). Further increase in the percent bioaccessible iron was evident when the food grains were microwave- cooked; the percent increase in bioaccessibility being as high as 69% in cowpea. Microwave cooking decreased iron bioaccessibility in sorghum, maize, whole chickpea and French bean by 44, 12, 23 and 60% respectively. Incidentally, the percent bioaccessibility of both zinc and iron from pressure-cooked cereals was similar.

Between these two heat-processing methods employed here, pressure-cooking is the commonly adopted practice in Indian households. As evident from Table-9, the actual bioaccessible zinc content (mg/100g) of pressure-cooked cereals ranged from 0.05 (finger millet) to 0.21 (sorghum), while that of iron ranged from 0.16 (rice and finger millet) to 0.47 (sorghum). Although the percent bioaccessibility of iron from pressure-cooked rice is the highest, it still cannot be considered as the best provider of this mineral among cereals, because of the low inherent iron content. Sorghum, which is the staple of a majority of the rural population, appears to be the best provider of both zinc and iron (Table 2). The bioaccessible zinc content (mg/100g) from pressure-cooked pulses as enumerated in Table 2 ranged from 0.36 in whole green gram to 1.18 in decorticated chickpea, whereas bioaccessible iron from these pressure-cooked pulses ranged from 0.11 (whole green gram) to 0.70 (French beans). Thus, each one of these pulses seems to be better providers of zinc than of iron, despite consistently higher inherent iron content.

Fig.24 Influence of heat processing on iron bioaccessibility from cereals

Values are mean ± SEM of 5 replicates

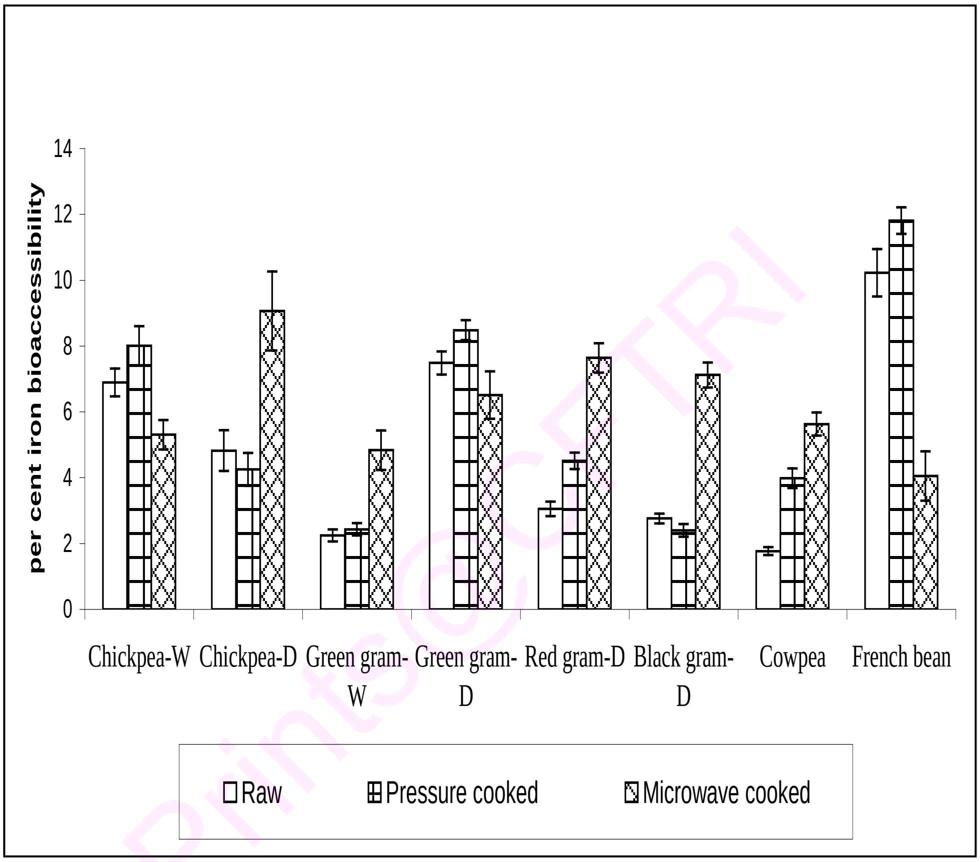


Fig.25 Influence of heat processing on iron bioaccessibility from pulses.

Values are mean ± SEM of 5 replicates.

W: Whole grain D: Decorticated grain

Table 9: Zinc and iron bioaccessibility from pressure– cooked food grains

Food grain	Bioaccessible zinc	Bioaccessible iron
Cereals		
Rice	0.10	0.16
Wheat	0.14	0.27
Finger millet	0.05	0.16
Sorghum	0.21	0.47
Maize	0.13	0.37
Pulses		
Chickpea (whole)	0.48	0.40
Chickpea (decorticated)	1.18	0.19
Green gram (whole)	0.36	0.11
Green gram (decorticated)	0.61	0.33
Red gram decorticated	0.87	0.22
Black gram (decorticated)	0.60	0.15
Cowpea	1.07	0.19
French bean	0.95	0.70

Values expressed as mg/100g, are average of five independent determinations

Among the pulses examined, decorticated chickpea, cowpea, French bean and red gram are among the best sources of bioaccessible zinc. French bean stands out among pulses as far as iron bioaccessibility is concerned, whereas the rest of the pulses have bioaccessible iron content to cereals. It is generally believed that heat processing improves the digestibility and bioavailability of both macro- and micronutrients. However, this trend was not evident in the case of zinc bioaccessibility from a majority of food grains in the present study. The differences between zinc and iron dialyzabilities from food grains can be attributed to the different chemical forms of these metals (valence states), different physicochemical environment, and their possible different localizations in the grains. These minerals are linked or complexed to other different constituents. The types of linkages and associated constituents could be different for zinc and iron.

The decrease in zinc bioaccessibility on heat treatment of the tested food grains in the present study could be attributed to interactions of zinc with proteins, and / or other food components thereby hindering its absorption. Although the zinc bioavailability from raw pulses was higher relative to cereals, the negative effect of cooking was also higher in the case of pulses. Whether this observation that the decrease in zinc bioaccessibility was more prominent in the case of pulses upon heat possessing, is attributable to the higher amounts of protein present in them, remains to be evidenced. Cooking of food grains, which generally improves protein digestibility, has not resulted in improved absorbability of zinc, unlike that of iron. Carbonaro *et al* (1995) have reported that iron bioavailability was compromised as a result of impaired protein digestibility, while that of zinc did not seem to be affected. There was no difference in zinc dialysability from two globulins with different digestibility extracted from white beans. The authors inferred that the main determinant of zinc dialysability was probably amino acid composition of the protein, rather than its digestibility. This suggested that interaction of zinc with protein was more specific compared to that of iron. Differences, if any, in the nature of interaction of the two metals with food proteins upon heat processing, remain to be understood.

Cooking presumably modifies the seed composition, in turn influencing the zinc and iron dialysability. Cooking has been reported to result in phytate reduction in food grains. The changes in Zn and Fe dialysability caused by cooking as evidenced here cannot be fully explained by the changes occurring in any single constituent. Interactions with the protein matrix may play an important role as far as the potential mineral availability is concerned. Further studies are necessary to better understand the changes in food matrix induced by cooking which mostly influence zinc and iron availability. The differences in *in vitro* availability that exist between zinc and iron present in food grains could be due to their different chemical forms, differences in their association with other grain constituents, and differences in their localizations within the grain.

The *in vitro* method employed here for the estimation of mineral (Zn and Fe) availability is based on simulation of gastro-intestinal digestion and estimation of the proportion of the nutrient convertible to an absorbable form in the digestive tract, by measuring the fraction that dialyses through a membrane. The dialysability of a mineral gives a fair estimate of its availability for absorption *in vivo*. Such *in vitro* methods are rapid, simple and inexpensive. However, it should be remembered that the results from *in vitro* methods are relative rather than absolute estimates of mineral absorbability, since they are not subjected to the physiological factors that can affect bioavailability (Sebastia *et al*, 2001). Such relative estimates of the mineral availability in terms of *in vitro* zinc and iron dialysability obtained in the present investigation on the comparative zinc bioavailability values for different food grains, and the influence of cooking on the same are still valid and suffice to form a strategy to derive maximum mineral availability.

SUMMARY

Several food grains commonly consumed in India were screened for zinc content and its bioaccessibility from the same was determined by equilibrium dialysis employing an *in vitro* simulated digestion procedure. The food grains examined included the cereals - rice (*Oryza sativa*), finger millet (*Eleusine coracana*), wheat (*Triticum aestivum*), maize (*Zea mays*), sorghum (*Sorghum vulgare*), and the pulses - chickpea (*Cicer arietinum*), green gram (*Phaseolus aureus*), red gram (*Cajanus cajan*), black gram (*Phaseolus mungo*), cowpea (*Vigna catjang*), and French bean (*Phaseolus vulgaris*). The total zinc content of cereals ranged from 1.08 mg / 100 g in rice to 2.24 mg/ 100 g in sorghum. The zinc content of pulses was between 2.03 mg/

100 g (whole chickpea) and 2.68 mg/ 100 g (decorticated chickpea). The bioaccessibility of zinc from pulses (27 - 56%) was generally higher than that from cereals (5.5 - 21.4%). The iron content of cereals ranged from 1.32 mg% in rice to 6.51 mg% in sorghum, while that of pulses ranged from 3.85 mg% in decorticated green gram to 6.46 mg% in black gram. Bioaccessibility of iron was almost similar from both cereals and pulses examined and ranged from 4.13 to 8.05% in cereals and from 1.77 to 10.2 % in pulses.

The bioaccessibility values of zinc and iron were viewed in relation to the inherent concentrations of phytic acid, calcium, tannin and fibre. Phytic acid content of these food grains ranged from 160 to 630 mg %. A significant negative correlation between inherent phytate and zinc bioaccessibility value was inferred in the case of pulses. However, the same was not evident when the bioaccessibility value was viewed in relation to phytate: zinc molar ratios. In the case of iron, phytic acid content of the cereal grains produced a proportionate and statistically significant negative influence on its bioaccessibility. The calcium content of the food grains tested ranged from 7.2 mg % in maize, to 325 mg % in finger millet. This inherent calcium had a negative influence on zinc bioaccessibility in cereals. Zinc bioaccessibility from the food grains was also negatively influenced by [phytate] x [calcium] / [zinc] molar ratio, which however was not statistically significant. Such an inverse relationship of calcium or of [phytate] x [calcium] / [iron] molar ratio was not evident on iron bioaccessibility.

Among the food grains tested, finger millet had the highest amount of tannin (2.12 g %) and similar high amounts were present in French beans (3.08 g %) and cowpea (2.21 g %). Tannin did not have any significant influence on zinc and iron bioaccessibility from cereals and pulses. Among the test grains, legumes (especially whole) had higher dietary fibre content. While both insoluble and soluble fractions of the dietary fibre in the food grains generally interfered with zinc bioaccessibility, the insoluble fraction alone had this effect on iron bioaccessibility. Soluble fraction, on the other hand, had the opposite effect on iron bioaccessibility, more so in the case of cereals.

The lower collective negative influence of the various inherent factors on zinc bioaccessibility from pulses is consistent with the higher values of the same in these grains, relative to cereals. The extent of collective influence of the various inherent factors on iron bioaccessibility from cereals and pulses was essentially similar.

The negative correlation of inherent phytic acid with zinc and iron bioaccessibility was supported by enhanced bioaccessibility of these minerals observed upon partial removal of phytate from the food grains by treatment with fungal phytase. Exogenous phytic acid showed a marginal decreasing effect on zinc bioaccessibility from the food grains studied, which was however not proportionate to the level added. Iron bioaccessibility values, however, were profoundly enhanced from all the food grains tested.

Influence of heat processing on the *in vitro* availability of zinc from these cereals and pulses was also examined. In general, zinc bioaccessibility from these food grains was considerably reduced upon domestic cooking (pressure-cooking or microwave heating). Zinc bioaccessibility was reduced to nearly half in rice, wheat, chickpea and green gram. Iron bioaccessibility on the other hand, was significantly enhanced generally from all the food grains studied upon heat treatment. Thus, heat treatment of grains produced contrasting effect on zinc and iron bioaccessibility.

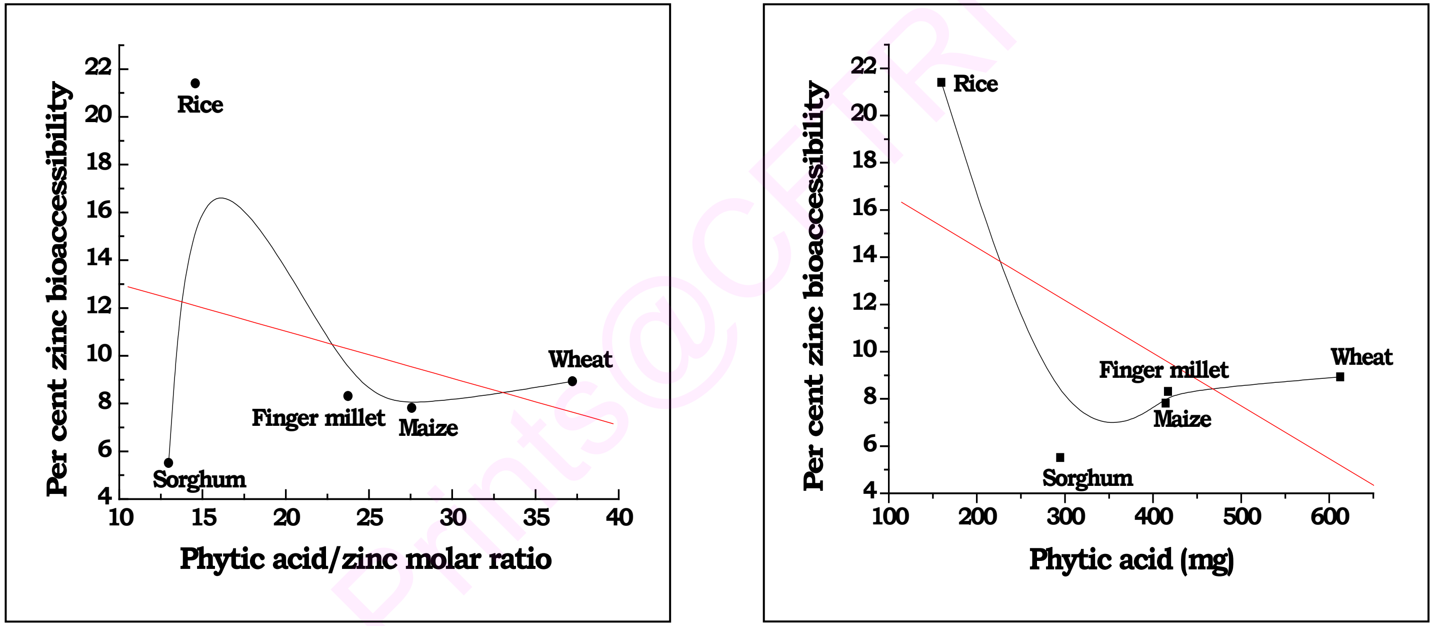


Fig.5 Correrelation of zinc bioaccessibility of cereals with inherent phytic acid.

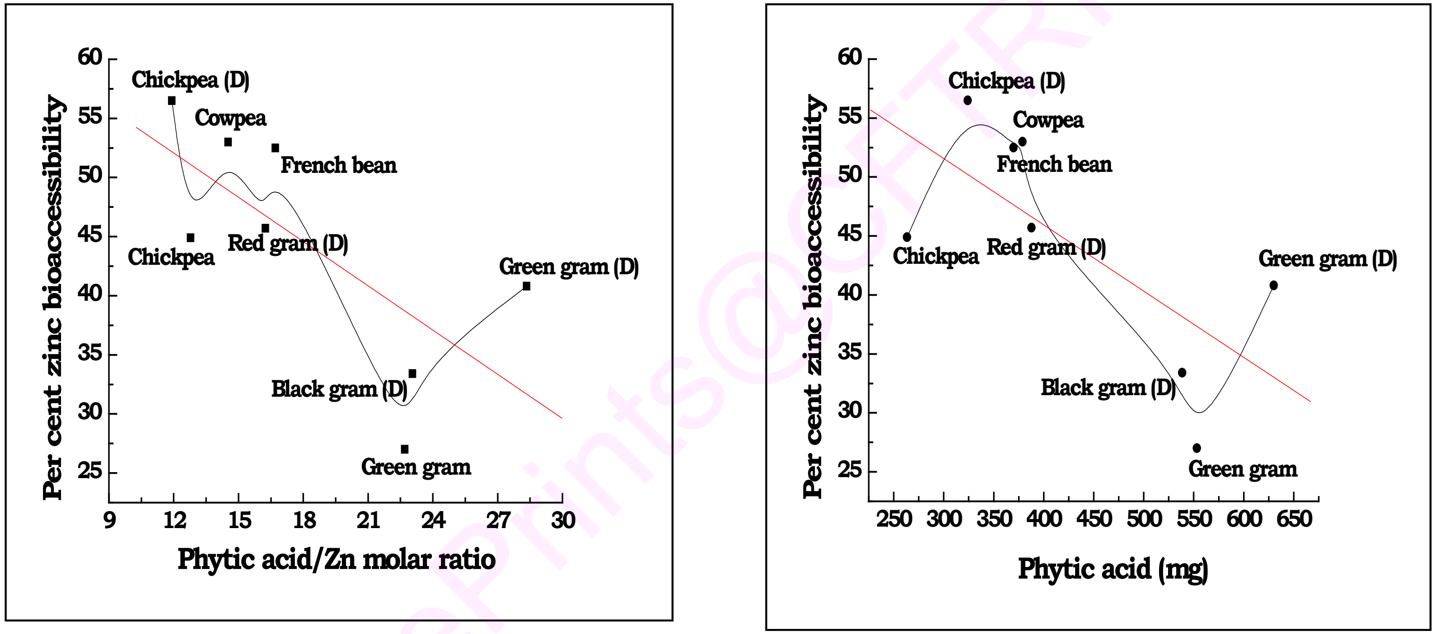


Fig.6 Correlation of zinc bioaccessibility of pulses with inherent phytic acid

D: decorticated grain

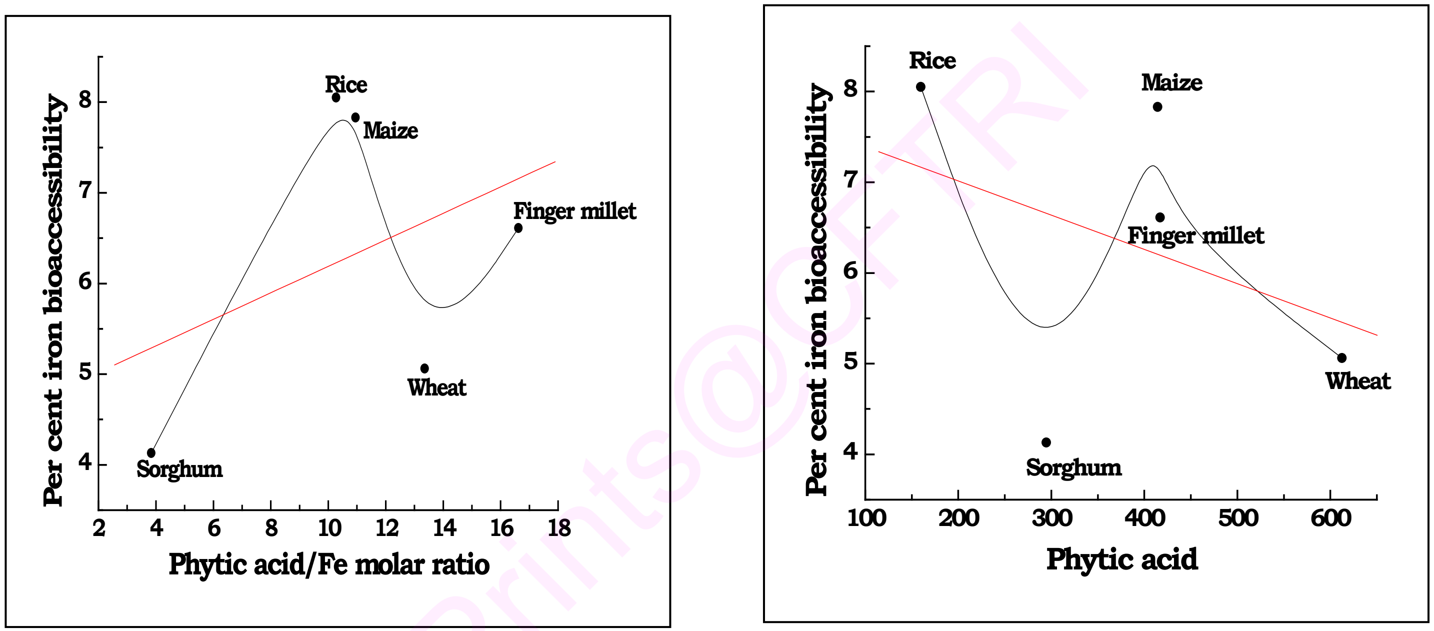


Fig.7 Correlation of iron bioaccessibility of cereals with inherent phytic acid

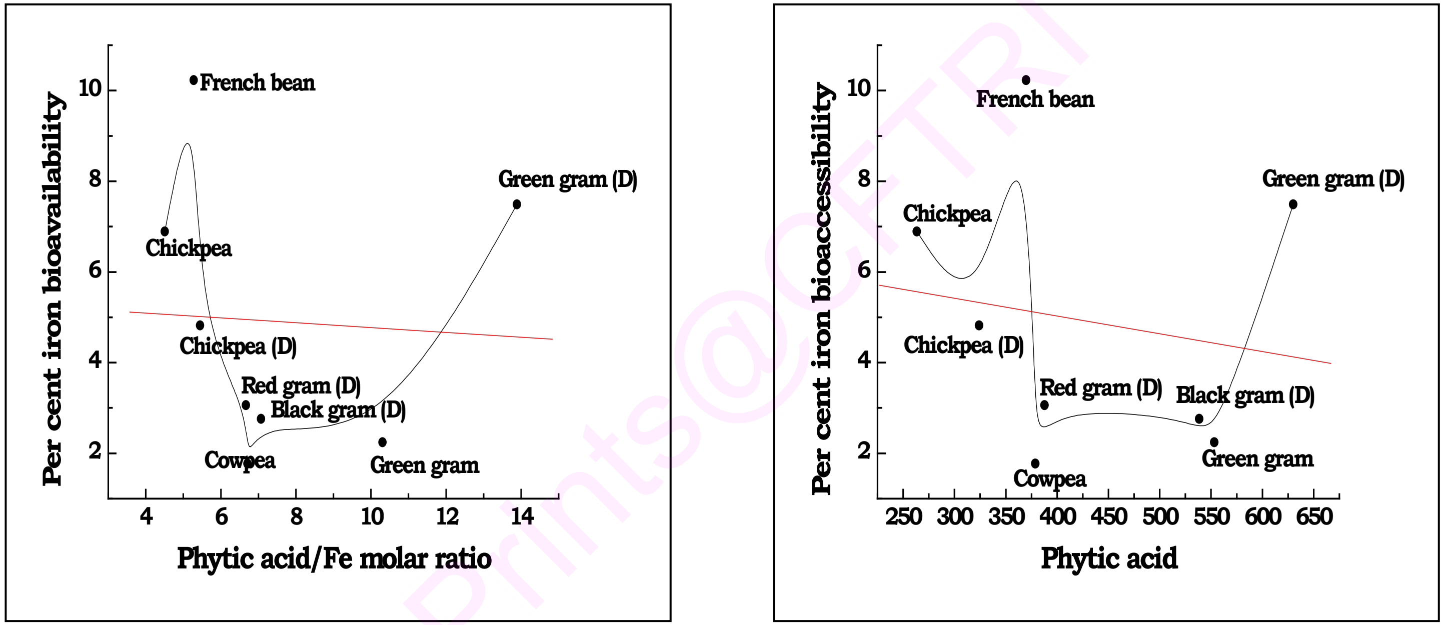
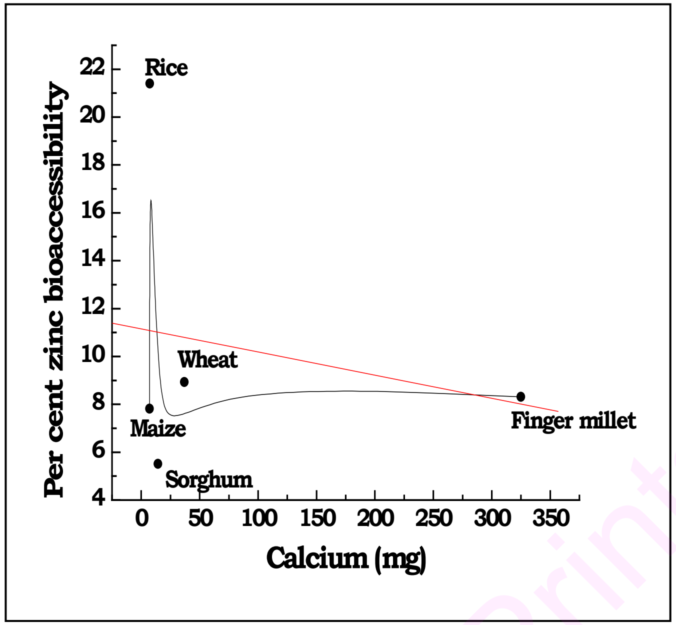


Fig.8 Correlation of iron bioaccessibility of pulses with inherent phytic acid

D: decorticated grain



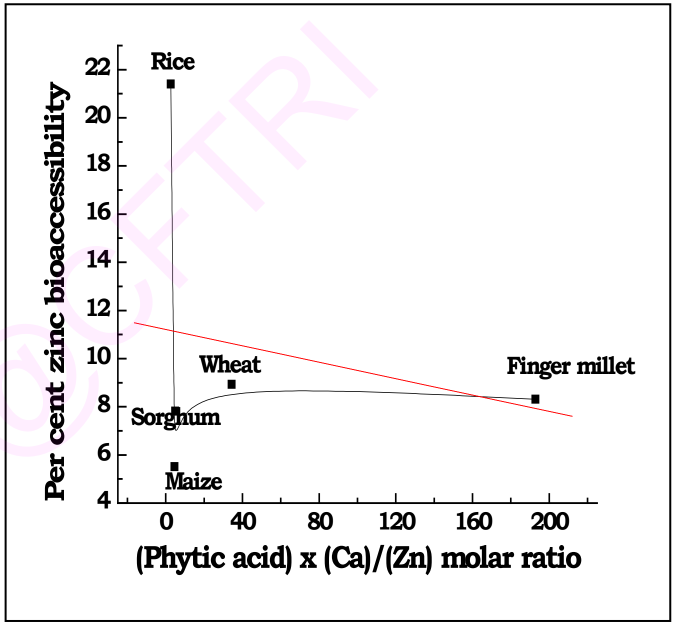


Fig.9 Correlation of zinc bioaccessibility of cereals with inherent calcium

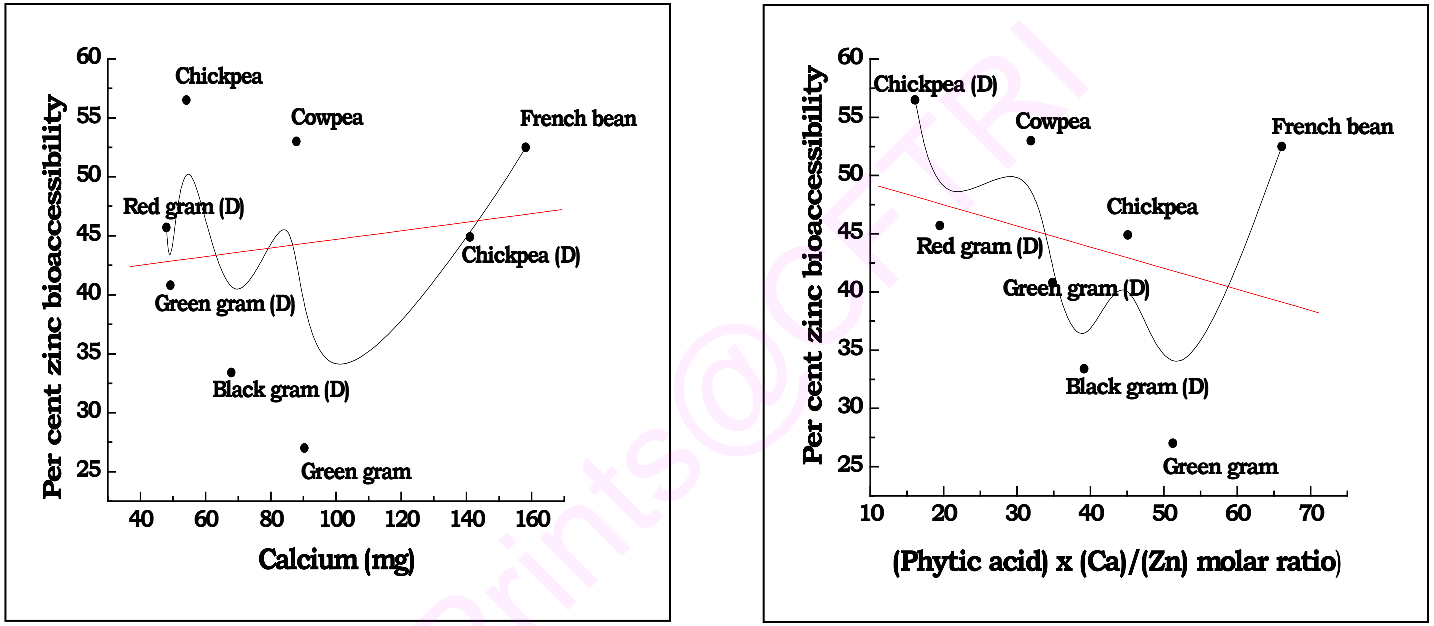


Fig.10 Correlation of zinc bioaccessibility of pulses with inherent calcium

D: decorticated grain

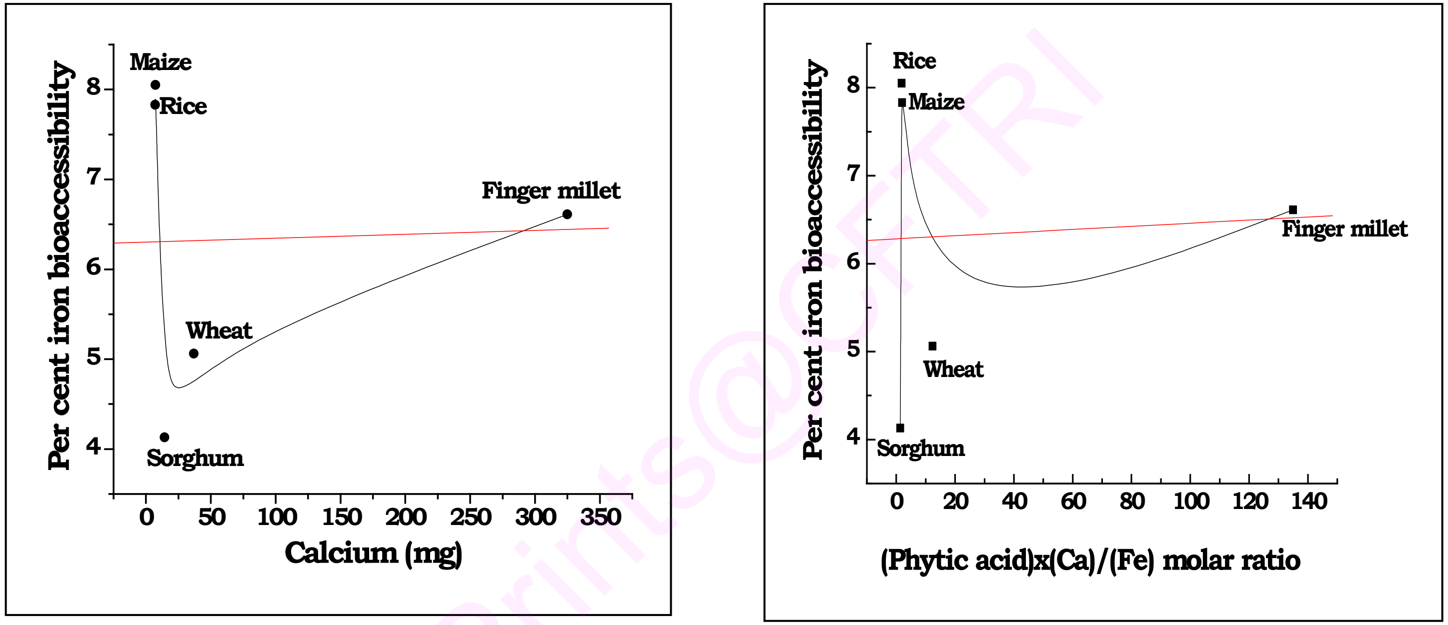


Fig.11 Correlation of iron bioaccessibility of cereals with inherent calcium

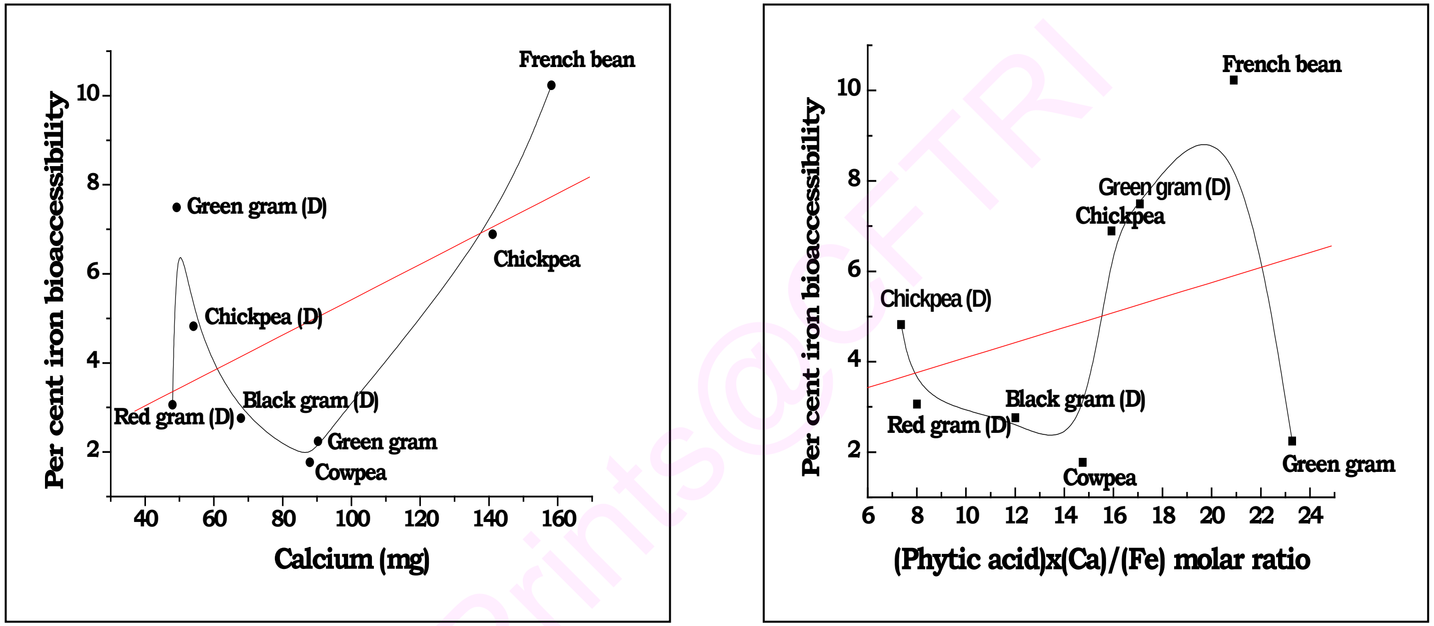


Fig.12 Correlation of iron bioaccessibility of pulses with inherent calcium

D: decorticated grain

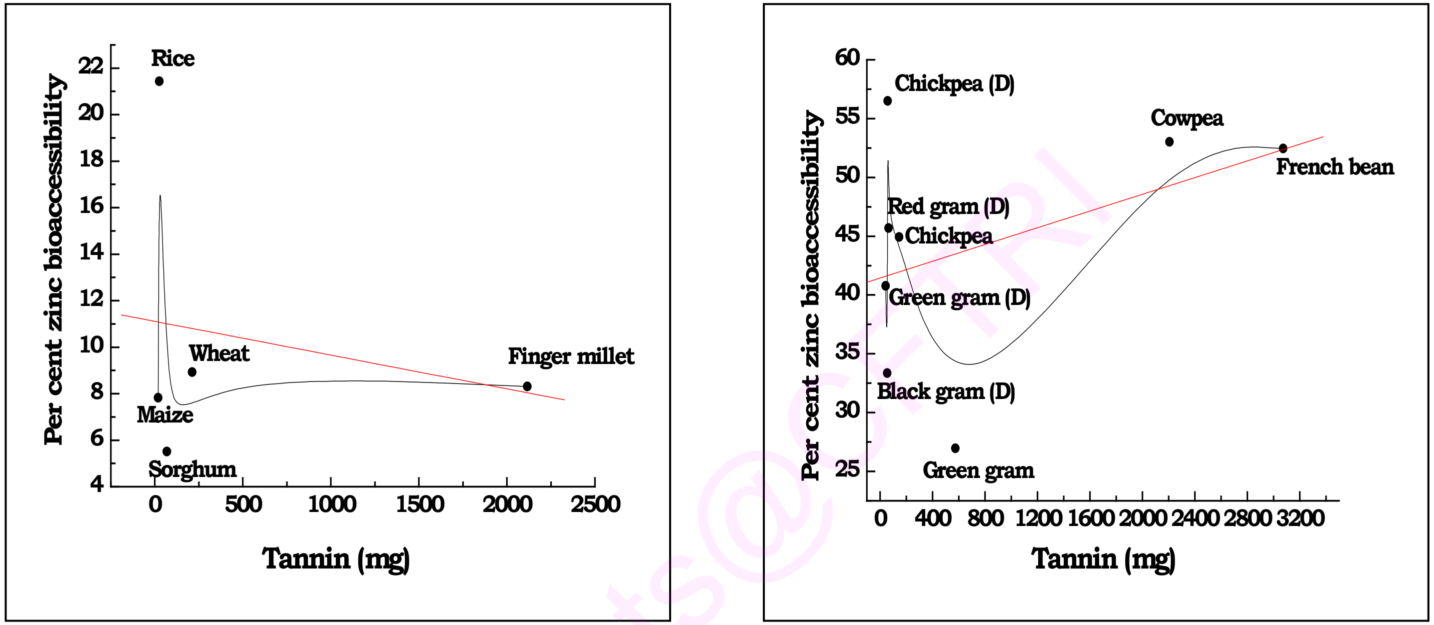


Fig.13 Correlation of zinc bioaccessibility of cereals and pulses with inherent tannin

D: decorticated grain

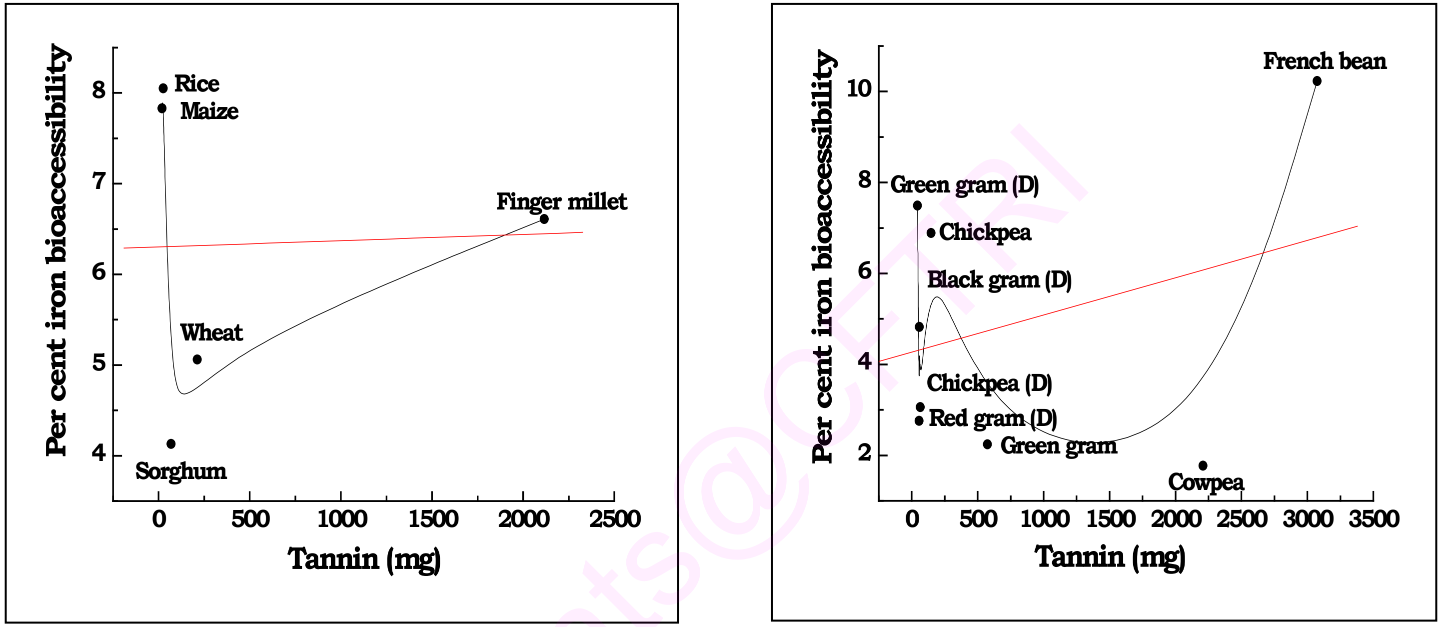


Fig.14 Correlation of iron bioaccessibility of cereals and pulses with inherent tannin

D: decorticated grain

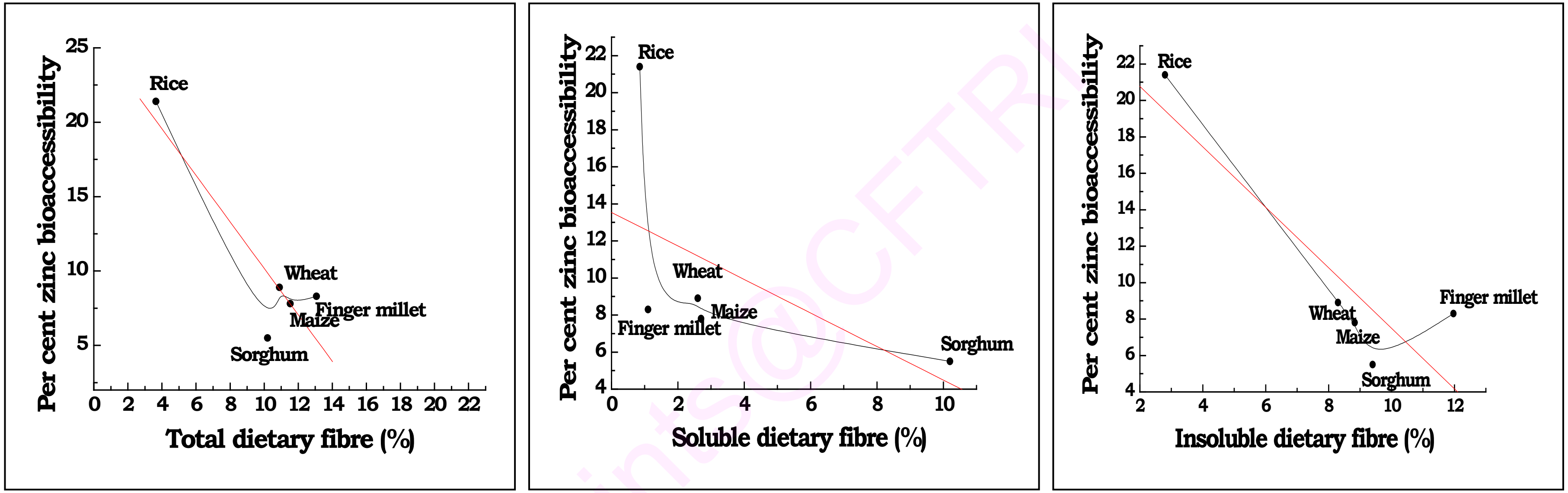


Fig.15 Correlation of zinc bioaccessibility of cereals with inherent dietary fibre

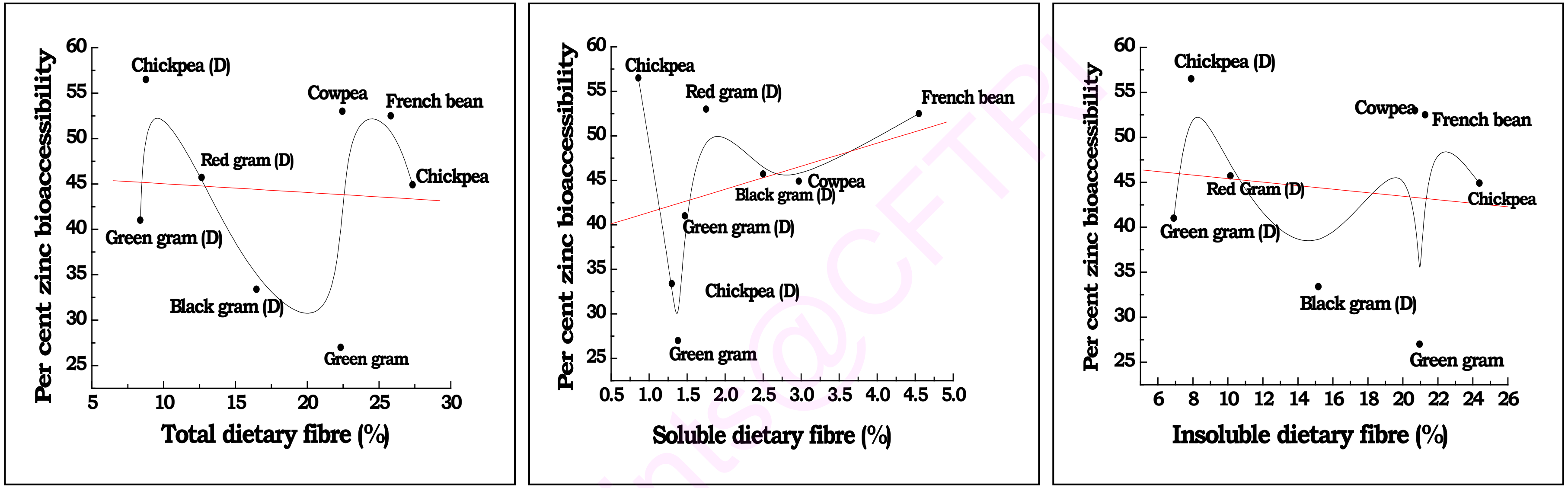


Fig.16 Correlation of zinc bioaccessibility of pulses with inherent dietary fibre

D: decorticated grain

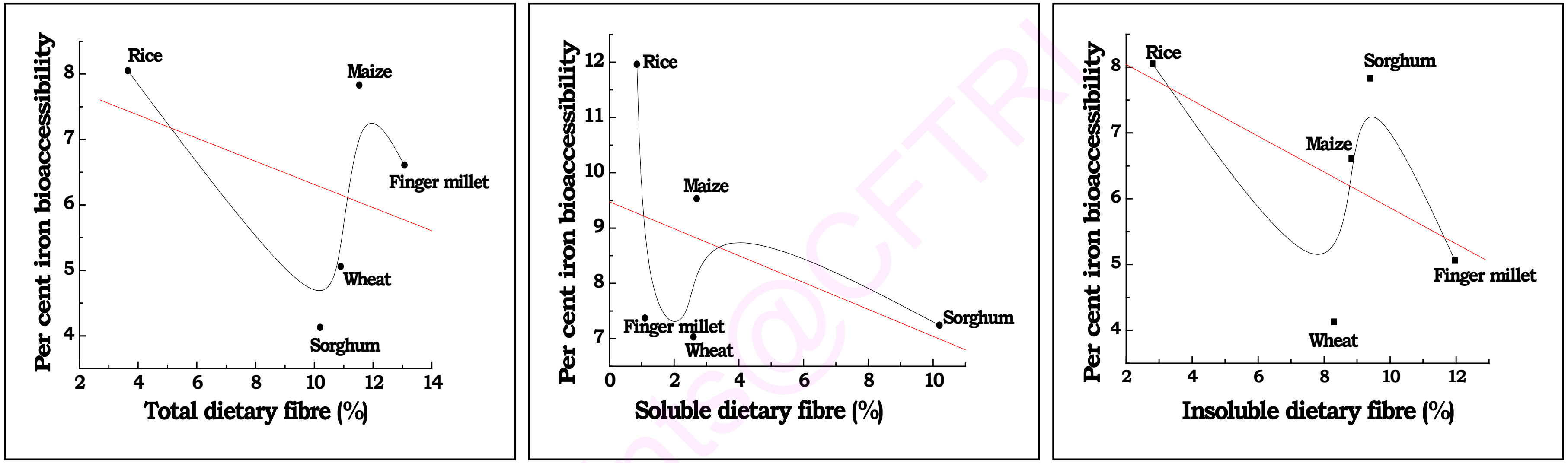


Fig.17 Correlation of iron bioaccessibility of cereals with inherent dietary fibre

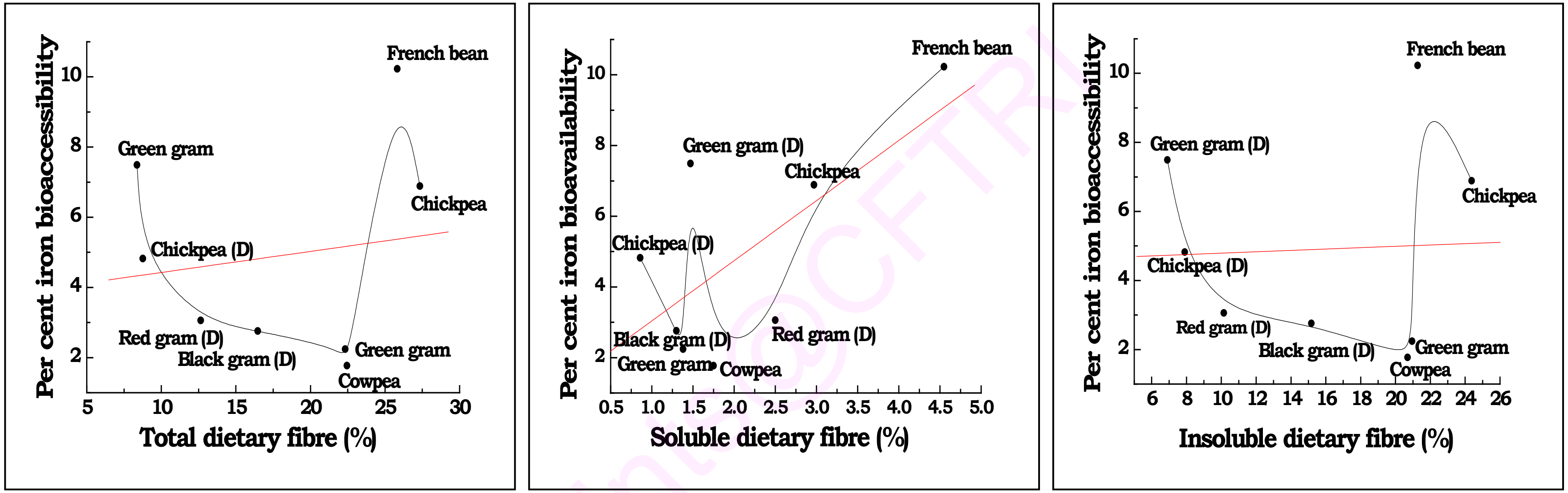


Fig.18 Correlation of iron bioaccessibility of pulses with inherent dietary fibre

D: decorticated grain

CHAPTER – III

INFLUENCE OF EXOGENOUS IRON, CALCIUM, PROTEIN AND COMMON SALT ON THE BIOACCESSIBILITY OF ZINC FROM FOOD GRAINS

Influence of Exogenous Iron, Calcium, Protein and Common Salt on the Bioaccessibility of Zinc from Food Grains

INTRODUCTION

Zinc and iron bioaccessibility values in cereals and pulses commonly consumed in India determined by us are reported in chapter II. Considerable differences in the extent of bioaccessibility of these two minerals from cereals and pulses were evidenced. Apart from inherent modulators of mineral absorption such as phytate, tannin and insoluble dietary fibre, minerals that are similar in chemical configuration are likely to compete with each other at the site of absorption, thus coming in the way of their bioavailability (Gibson, 1994)). When we viewed the zinc bioaccessibility from cereals in relation to zinc: iron molar ratio, there was a slight trend towards increased bioaccessibility with increased zinc: iron molar ratio (Chapter II).

Although it is inferred that iron probably does not affect zinc absorption at the molar ratios inherent in the food grains, pharmacological supplements of the same may have a bearing on the bioavailability of zinc. Supplements containing iron and multiple trace elements and minerals are used by millions of people world-wide. It is also a common practice to take iron supplements during pregnancy in the developing countries. It is important to provide detailed information regarding safe upper intake limits for supplements to minimize adverse effects on mineral absorption caused by mineral – mineral interactions. It has long been recognized that iron metabolism interacts with the metabolism of several other micronutrients such as zinc and copper. Iron was shown to decrease zinc absorption in humans in a dose dependent way when given in a water solution but not when given with the meal (Valberg *et al*, 1985; Sandstrom *et al*, 1985). Iron supplements have been reported to decrease zinc absorption in pregnant women (Simmer & Thompson, 1985), and lower serum zinc concentrations were observed in teenage pregnant women taking daily multi-vitamin supplements containing 18 mg iron than in those taking multivitamin supplements without any iron (Dawson *et al*, 1989).

Inherent calcium had a negative influence on zinc bioaccessibility in cereals (Chapter II). While calcium has made a difference in zinc accessibility at the levels normally inherent in the grain especially in cereals, higher doses of the same as encountered in therapeutic supplementation of calcium, is expected to further modulate zinc bioaccessibility from food grains. In this context, the influence of exogenously added iron and calcium at a level up to four times the inherent concentration on the bioaccessibility of zinc from selected food grains was examined.

Our previous observation that: (1) per cent zinc bioaccessibility was higher in pulses than in cereals in spite of generally higher presence of factors such as phytate, tannin, calcium and insoluble dietary fibre; and (2) the overall effect of such inherent factors on zinc bioaccessibility from the grain was more prominent in the case of cereals than in pulses, suggests that inherent factors other than the above such as protein of which pulses are a richer source, may have a modulatory role on zinc bioaccessibility. In addition, heat– treatment of grains produced contrasting effects on zinc and iron bioaccessibility, namely, iron bioaccessibility was enhanced upon cooking, while that of zinc was generally reduced in both cereals and pulses (Chapter II). Such a contrasting effect of heat-treatment on zinc and iron bioaccessibility could probably be attributed to differences in their association with the protein constituent of the grain. In this context, the influence, if any, of exogenous protein, on zinc and iron bioaccessibility from representative cereals was also examined. Common salt, which is a regular ingredient of our cookery, can alter the solubility characteristics of food proteins. Hence, influence of common salt along with exogenous protein on the bioaccessibility of zinc and iron from food grains has also been specifically examined.

MATERIALS AND METHODS

Materials:

Cereals - rice (*Oryza sativa*), sorghum (*Sorghum vulgare),* and wheat (*Triticum aestivum,* and pulses - chickpea (*Cicer arietinum*) - whole and decorticated and green gram (*Phaseolus aureus*) – decorticated were procured locally, cleaned and used for the studies. Pepsin, pancreatin (porcine) and bile extract (porcine) were obtained from Sigma Chemical Co., USA. All chemicals used here were of analytical grade. Triple distilled water was employed during the entire study. Acid-washed glassware was used throughout the study.

***Zinc, iron and calcium*:**

Grain samples were ground finely and ashed in a muffle furnace at 550^{o}C for 5 h and the ash was dissolved in conc. HCl. Zinc, iron and calcium content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of measurements was performed using commercial standards. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron. In the case of calcium, lanthanum chloride was added to the mineral solution, to avoid interference from phosphate.

Bioaccessibility of zinc and iron:

Bioaccessibility of zinc and iron in the samples was determined by the *in vitro* method of Luten *et al* (1996) involving simulated gastrointestinal digestion with suitable modifications, as described in Chapter II.

Preparation of soy protein isolate:

Defatted soya flour (obtained by cold organic solvent extraction) was used for the preparation of soya protein isolate as described by Waggle *et al* (1989). The defatted flour was treated with sodium hydroxide to raise the pH to 8.5, and stirred for 2h on a magnetic stirrer; centrifuged and the pH of the supernatant was adjusted to 4.5 with HCl and allowed to stand for 1h. The protein precipitate separated by centrifugation was repeatedly washed with water to remove the acid, dissolved in water and the pH adjusted to 7.5 with NaOH. The solution was lyophilized. The resultant protein isolate contained 78% protein as determined by Kjeldahl method.

Heat processing of food grains:

Ten grams of the food grains were pressure-cooked in 30 ml of triple distilled water for 10 min (15 psi). The cooked samples were homogenized and used for the determination of mineral bioaccessibility as described above.

Studies with exogenous iron and calcium:

Rice and decorticated green gram were examined for the effect of exogenous iron, while rice and wheat were employed for the study on the influence of exogenous calcium. Exogenous iron (as ferrous sulphate) and calcium (as calcium carbonate) were added to the ground grains (10 g) at levels equal to, twice, thrice and four times the amount of these minerals present inherently. Bioaccessibility of zinc and iron from these samples was determined as described above.

Studies on influence of exogenous protein:

The food grains examined in this context were rice, and sorghum with a protein content of 6.8 and 10.4%, respectively. Soy protein isolate prepared as described above was added to the powdered grain samples in such quantities to result in a protein content of 20 g%. The samples were also subjected to pressure cooking, and zinc and iron bioaccessibility from both raw and cooked samples were determined as described above. In a parallel set of samples, sodium chloride was added at 5% level in addition to the protein isolate, and the mineral bioaccessibility was determined in both raw and cooked forms.

Influence of exogenous sodium chloride:

Whole and decorticated chickpea, rice and sorghum were employed for the studies on the effect of exogenous sodium chloride on bioaccessibility of zinc and iron. Sodium chloride was added to the ground samples at 5% level. The above samples were also subjected to pressure-cooking as described above. Zinc and iron bioaccessibility were determined in both raw and cooked samples.

Determination of zinc and iron bioavailability in these variations of food samples, as well as all other chemical analyses was carried out in five replicates.

Statistical analysis:

Statistical analysis of analytical data was done according to Snedecor and Cochran (1977).

RESULTS AND DISCUSSION

Influence of exogenous iron on mineral bioaccessibility:

Fig.1 presents the zinc bioaccessibility from green gram and rice as influenced by exogenously added iron. Exogenous iron had a negative influence on zinc bioaccessibility from green gram only when present at levels four and five times the normal content of the grain. The zinc: iron molar ratio varied from 0.484 to 0.097 in the green gram samples examined for zinc bioaccessibility. Thus, the effect of exogenous iron on zinc bioaccessibility from green gram was visible only when the zinc: iron molar ratio was 0.12 or lower. Effect of exogenous iron on zinc bioaccessibility was not seen in the case of rice because even at the highest concentration (five times the inherent content) of iron examined, the zinc: iron molar ratio was 0.14. The zinc: iron molar ratio varied from 0.697 to 0.14 in the rice samples examined for zinc bioaccessibility. The critical zinc: iron molar ratio is probably around 0.13 to exert an inhibitory influence on zinc bioaccessibility, which needs to be further substantiated.

Although we did not encounter severe limitations in zinc bioaccessibility from food grains tested in presence of exogenous iron, there is an indication that when the concentration of iron reaches a threshold level such that zinc: iron molar ratio is 1:8, zinc bioaccessibility will be compromised. Supplemental levels of iron are certainly likely to negatively influence zinc absorption. The interaction between iron and zinc absorption may be explained by competitive binding to the transporter protein (DMT-1), which is located at the apical membrane in the small intestine or by an iron induced decrease in the expression of DMT-1. Although the exact mechanism of iron absorption is still under investigation, it is clear that DMT-1 – mediated iron transfer accounts for most of the iron absorption from the intestine (Gunshin *et al*, 1997; Andrews, 1999). Antagonistic interactions between iron and zinc are most likely when high doses of supplemental zinc and iron are given in the absence of food, in the presence of low dietary intakes of these minerals, and when consumed between meals rather than with meals. Intakes of these minerals from dietary sources alone are unlikely to be high enough to compromise iron or zinc status (Prasad *et al*, 1978; Solomons, 1986; Rossander–Fulten *et al*, 1991; Valberg *et al*, 1985).

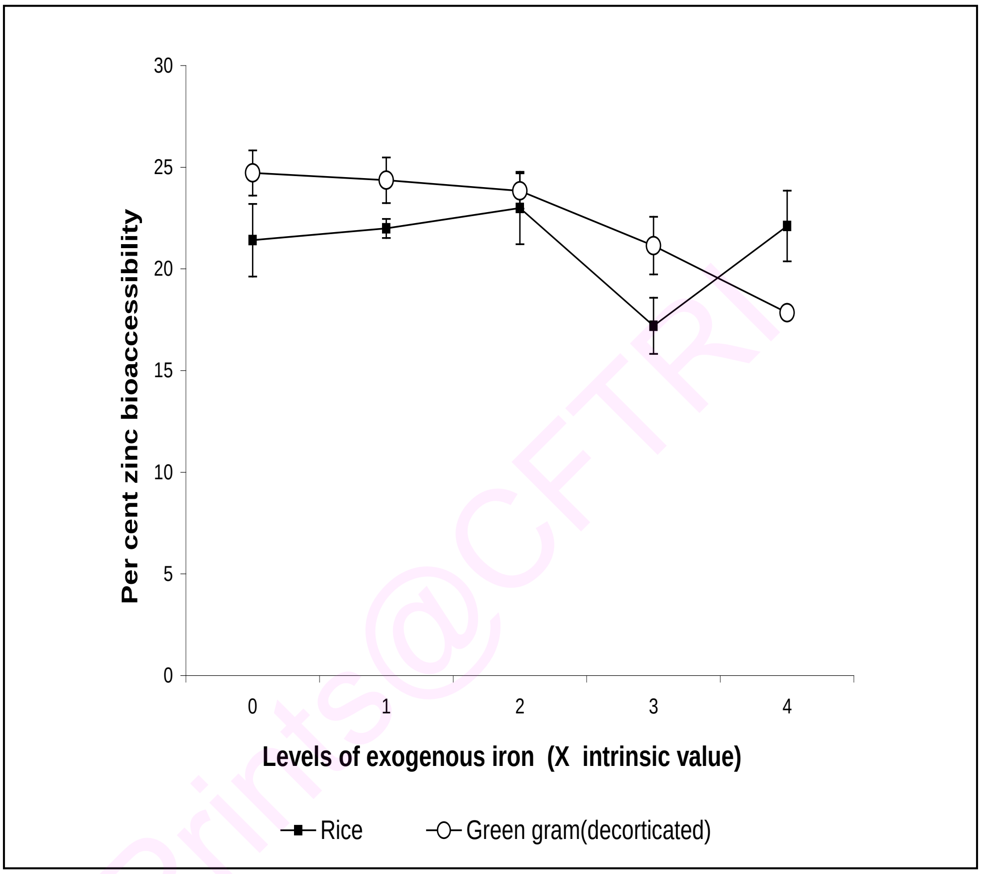


Fig.1 Zinc bioaccessibility from rice and green gram in presence of exogenous iron

Contrary to these reports, Davidsson *et al* (1995) demonstrated that iron fortification of foods is unlikely to affect zinc absorption by examining the effect of Iron fortification of bread (65 mg/kg), weaning cereal (500 mg/kg) and infant formula (12 mg/L) in human adults with the use of stable isotopes. No significant negative effect on zinc absorption was found. Similar results were obtained by Fairweather–Tait *et al* (1995), who studied the efironct of iron fortification of a weaning food on zinc absorption in infants with the use of stable isotopes.

Bioaccessibility of iron from the same samples of green gram and rice increased with increasing exogenous iron concentration up to four times the inherent iron content. Further increase in exogenous iron did not result any additional increase in iron bioaccessibility (Fig.2). Apparently, the inherent inhibitory factors of iron bioaccessibility present in the food grains tested did not have an impact on the bioaccessibility of the added iron. This could be attributed to the fact that exogenous iron was in the ferrous state, whose bioaccessibility is not usually influenced by dietary factors.

Influence of exogenous calcium on mineral bioaccessibility:

Zinc bioaccessibility values for rice and wheat in presence of exogenously added calcium are given in Fig.3. Exogenous calcium apparently did not negatively influence zinc bioaccessibility from rice when the calcium content was raised from 7.26 mg% to five times that, and from wheat when raised from 37 mg% to 185 mg%. Fig.4 presents the influence of exogenous calcium on the bioaccessibility of iron from the same samples of rice and wheat. As in the case of zinc bioaccessibility, exogenous calcium did not negatively influence iron bioaccessibility from the two food grains at the added levels. On the other hand, there was a slight increase in iron bioaccessibility with increasing calcium concentration from both the food grains. The absence of any negative influence of exogenous calcium on mineral bioaccessibility in the present experimental set-up (where there was no additional phytic acid) could be attributable to the fact that calcium brings about its negative effect only in association with phytate, while calcium *per se* does not have this effect (Spencer *et al*, 1984; Dawson-Hughes *et al*, 1986).

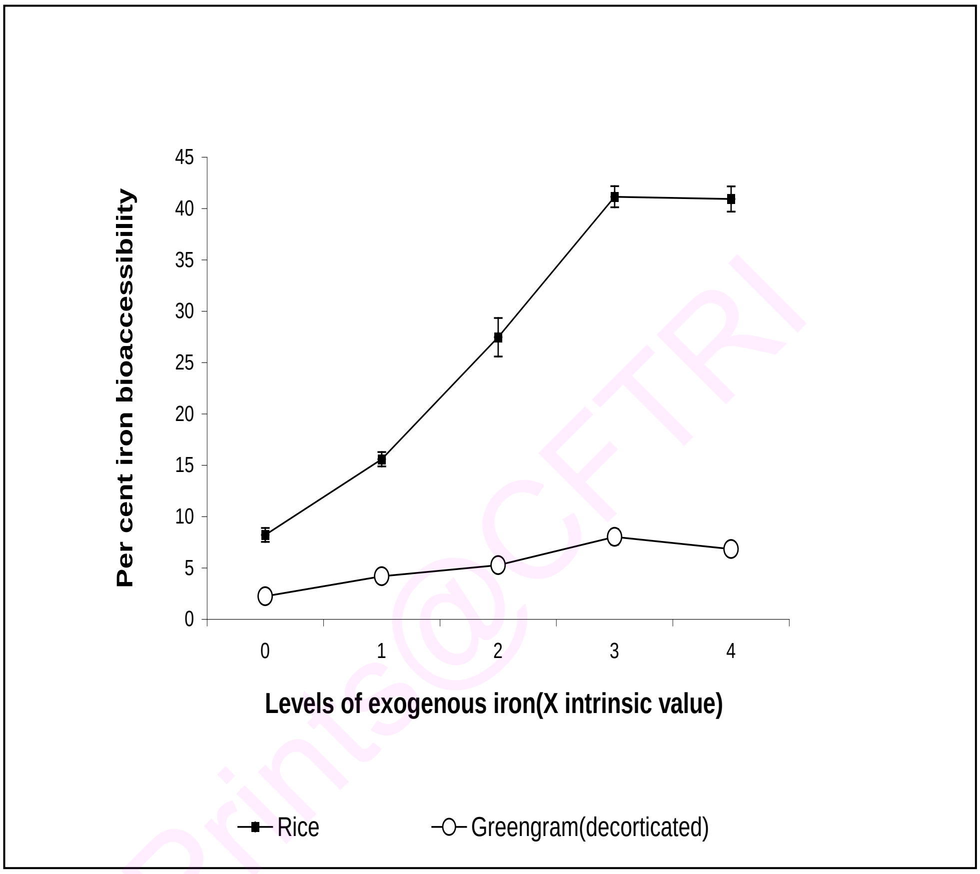


Fig.2 Iron bioaccessibility from rice and green gram in presence of exogenous iron

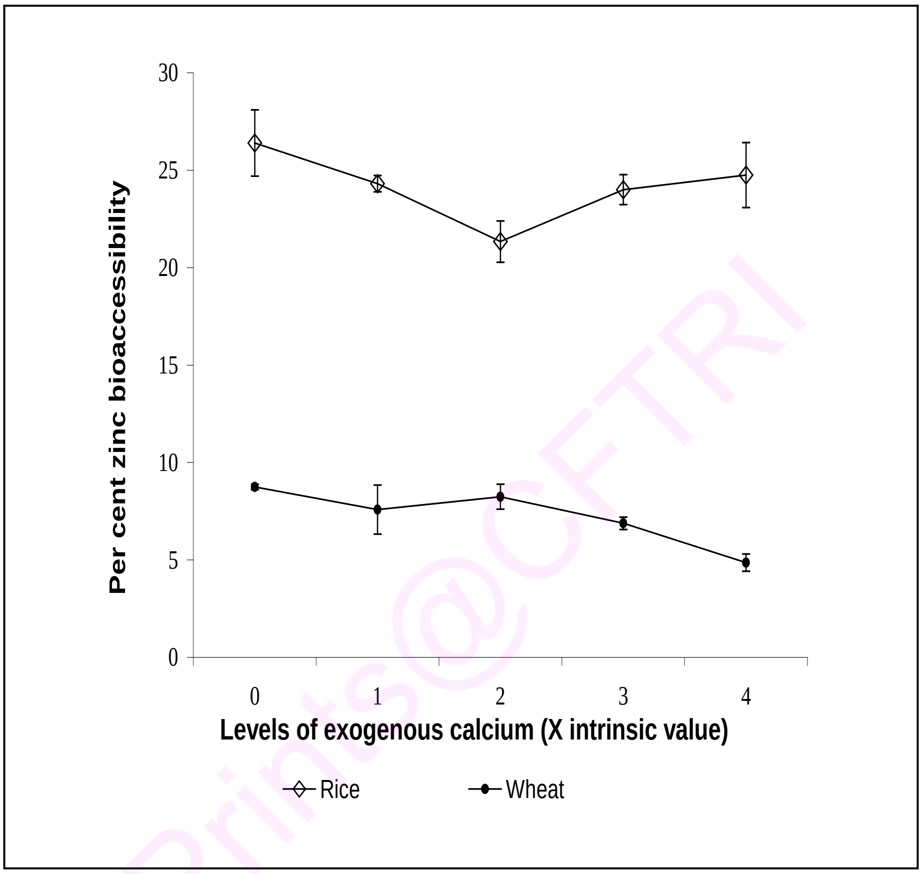


Fig.3 Zinc bioaccessibility from rice and wheat in presence of exogenous calcium

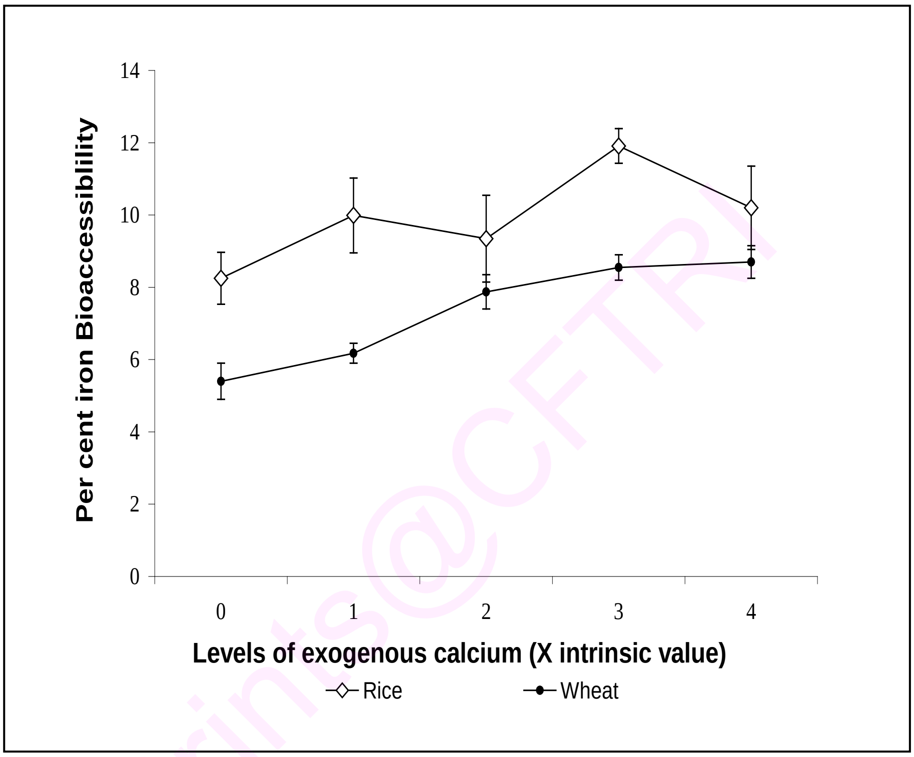


Fig.4 Iron bioaccessibility from rice and wheat in presence of exogenous calcium

Influence of exogenous soy protein isolate on mineral bioaccessibility:

In order to study the influence of exogenous protein on zinc and iron bioaccessibility, two representative cereals, namely rice and sorghum were selected and soy protein was added to bring the protein concentration of these two cereals (6.8 and 10.4%, respectively) to the levels normally encountered in pulses (around 20%). Figure 5 presents the influence of exogenous soy protein on zinc bioaccessibility from rice and sorghum. While soy protein did not have a positive influence on zinc bioaccessibility from rice either raw or cooked, the same had a significant positive influence on zinc bioaccessibility from both raw and cooked forms of sorghum (an increase of 49 and 91%, respectively). Sodium chloride which is normally present in our dietary, is likely to influence the solubility of food protein, and hence was examined in a parallel set of samples by inclusion at 5% level. As evident from Fig.5, Sodium chloride potentiated the positive effects of exogenous soy protein on zinc bioaccessibility from sorghum. It also had an enhancing effect on zinc bioaccessibility from raw and cooked rice, the effect being particularly significant in the case of the cooked grain.

Contrary to its effect on zinc bioaccessibility, exogenously added soy protein significantly decreased the bioaccessible iron value from rice and sorghum in both raw and cooked forms (Fig.6). The decrease in iron bioaccessibility brought about by soy protein was as high as 63 and 73% in the case of raw and cooked rice respectively, while a decrease of 30 and 16%respectively was seen in the case of raw and cooked sorghum. In presence of sodium chloride, however, the negative influence of exogenous soy protein was partially countered in the case of rice, whereas in the case of sorghum the same was completely checked (Fig.6).

The calculation of bioaccessibility values in this study has taken into consideration the amounts of respective minerals (zinc and iron) contributed by the exogenously added soy protein isolate. We have earlier documented 27 to 56% bioaccessibility of zinc from pulses relative to 5.5 to 21.4% from cereals despite the generally higher presence of factors such as phytate, tannin, calcium and insoluble dietary fibre (Chapter II). This suggests that inherent factors other than the above such as protein, of which pulses are a richer source, may have a modulatory role on zinc bioaccessibility. In the present study, raising the level of protein from the

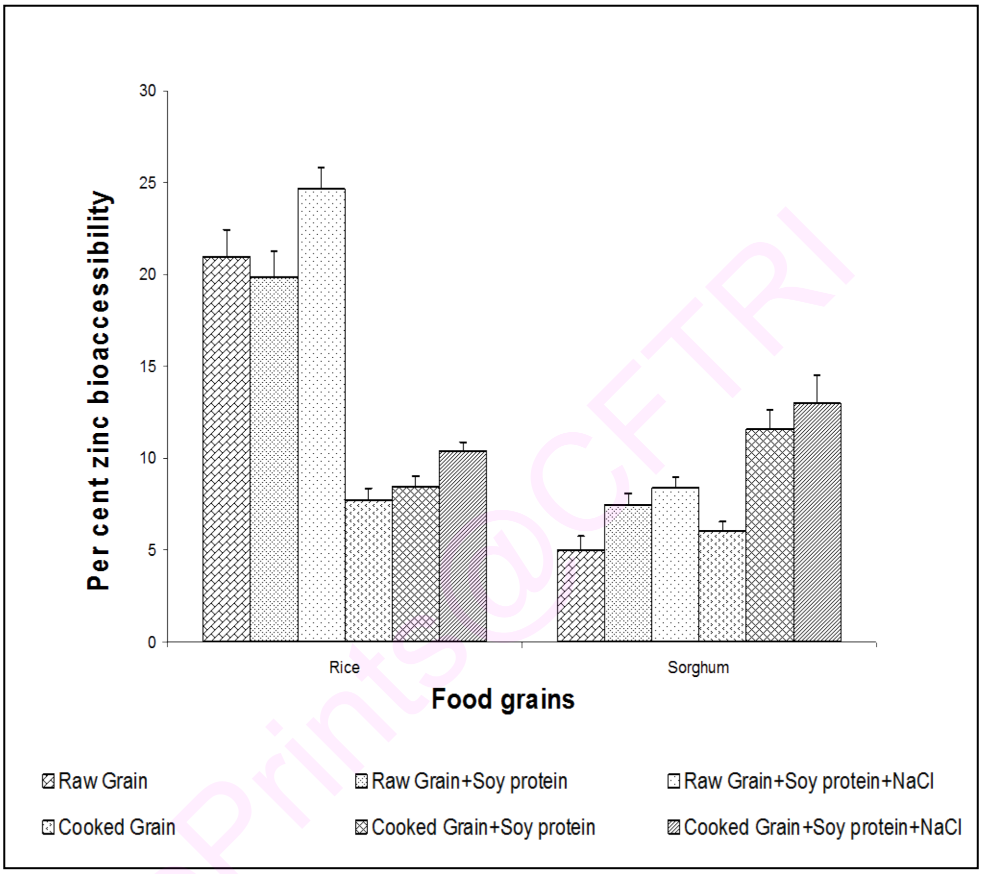


Fig.5 Zinc bioaccessibility from rice and sorghum in presence of exogenous protein

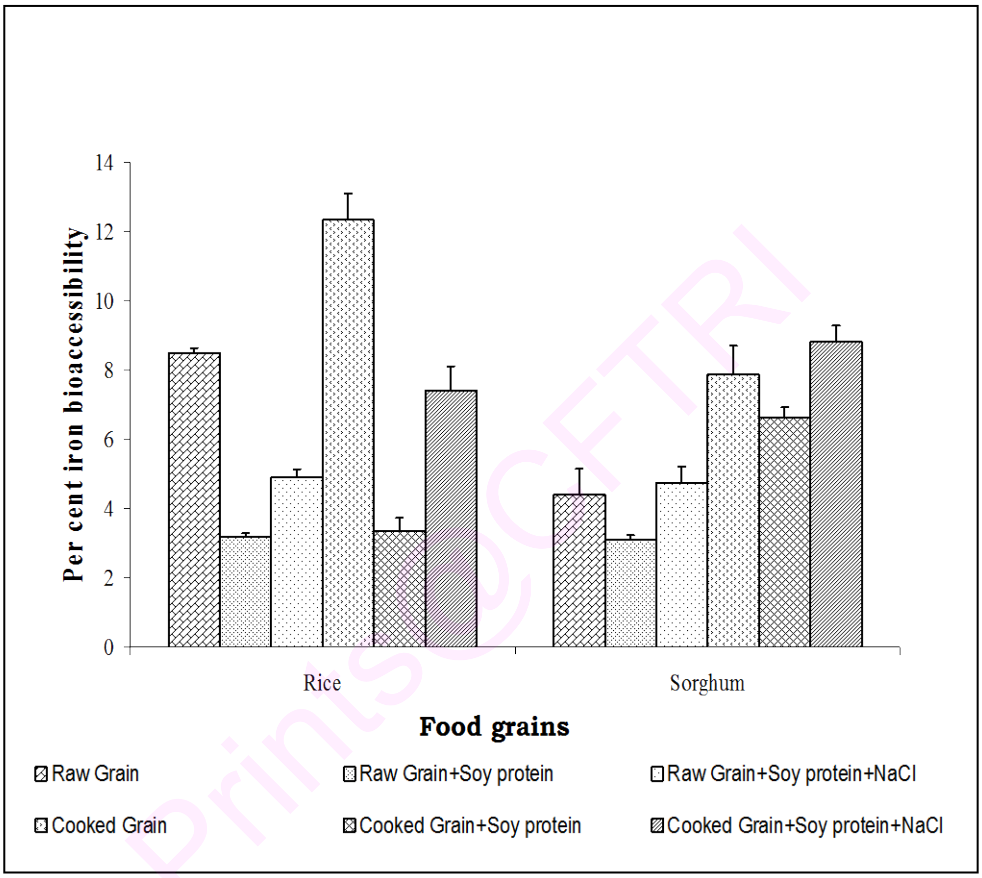


Fig.6 Iron bioaccessibility from rice and sorghum in presence of exogenous protein

representative cereals (rice and sorghum) to 20 g% (as in pulses) by the addition of exogenous soy protein did bring about an improvement in zinc bioaccessibility from sorghum, although this increased bioaccessibility value was still not comparable to that of pulses. On the other hand, exogenous soy protein had a negative influence on iron bioaccessibility from both the cereals tested. Thus the influence of exogenous soy protein on the bioaccessibility of zinc and iron from cereals appears to be contrasting. The negative influence of soy protein on iron bioaccessibility is probably attributable to rich amounts of phytic acid associated with it. We did evidence phytic acid concentration of 1.3 g% associated with the soy protein isolate used in this study. As reported in chapter II, the negative influence of phytic acid on iron bioaccessibility was more significant than on zinc bioaccessibility, especially from cereals. Whether proteins isolated from other legumes have effects similar to that of soy protein observed in this study with regard to influence on zinc and iron bioaccessibility needs to be examined. Several studies have already underlined the importance of the protein source for iron availability. Animal protein has been shown to counteract the inhibitory effect of phytate on zinc absorption (Sandstrom & Cederblad, 1980), attributable to amino acid release from the protein that keeps zinc in solution. Casein in milk has been shown to have a negative effect on zinc absorption (Sandstrom *et al*, 1983).

The amount of protein in a food is positively correlated to zinc absorption (Sandstrom *et al*, 1980). Fractional zinc absorption proportionately increased with protein content in humans provided with various protein sources and amounts (Sandstrom, 1992). Since protein is a major source of dietary zinc, increased dietary protein would lead to increased zinc intake and a higher bioavailability of the zinc provided.

Nelson and Potter (1980) found a relationship between protein digestibility and the release of protein bound iron. Keane and Miller (1984) suggested that the properties of undigested or partially digested protein influence iron availability. Hurrell *et al* (1989) reported that iron dialyzability increased according to the extent of milk protein hydrolysis. In a previous study (Lombardi-Boccia *et al*, 1994) we have pointed out that the degree of protein digestion and peptide composition might likely concur to modify iron availability from legumes. In our study, the inclusion of sodium chloride, which is likely to alter the solubility of food proteins, potentiated the positive influence of soy protein on zinc bioaccessibility and countered the negative effect of the same on iron bioaccessibility, which is in agreement with the reports that the digestibility of protein influences mineral bioavailability.

Studies on the effect of various protein sources are often confounded by the fact that the proteins often contain other constituents that may affect zinc absorption. Soy protein isolates, for example, normally contain considerable amounts of phytate.

Influence of exogenous sodium chloride on mineral bioaccessibility:

The influence of sodium chloride alone on zinc and iron bioaccessibility from two representative cereals and pulses was examined in a separate experiment. Influence of exogenous sodium chloride on zinc bioaccessibility from rice, sorghum, chickpea - whole and chickpea - decorticated is presented in Fig.7. Sodium chloride did not seem to positively influence the bioaccessibility of zinc from the food grains examined, except for rice and whole chickpea, both in their cooked forms. The extent of increase in rice was 25%, while that in chickpea was 93%.

Exogenously added sodium chloride significantly enhanced the bioaccessibility of iron from the majority of the food grains tested (Fig.8). The extent of increase was as high as 142% in the case of cooked rice, while it was 26% in its raw counterpart. The percent increase in iron bioaccessibility from raw and cooked sorghum was 27 and 34% respectively, while that in raw and cooked whole chickpea was 40 and 28%, respectively. Sodium chloride had no effect on the iron bioaccessibility values of both raw and cooked decorticated chickpea.

Thus, exogenously added sodium chloride brought about similar effects on zinc and iron bioavailability, the effect being limited to cooked rice and cooked whole chickpea in the case of zinc bioaccessibility.

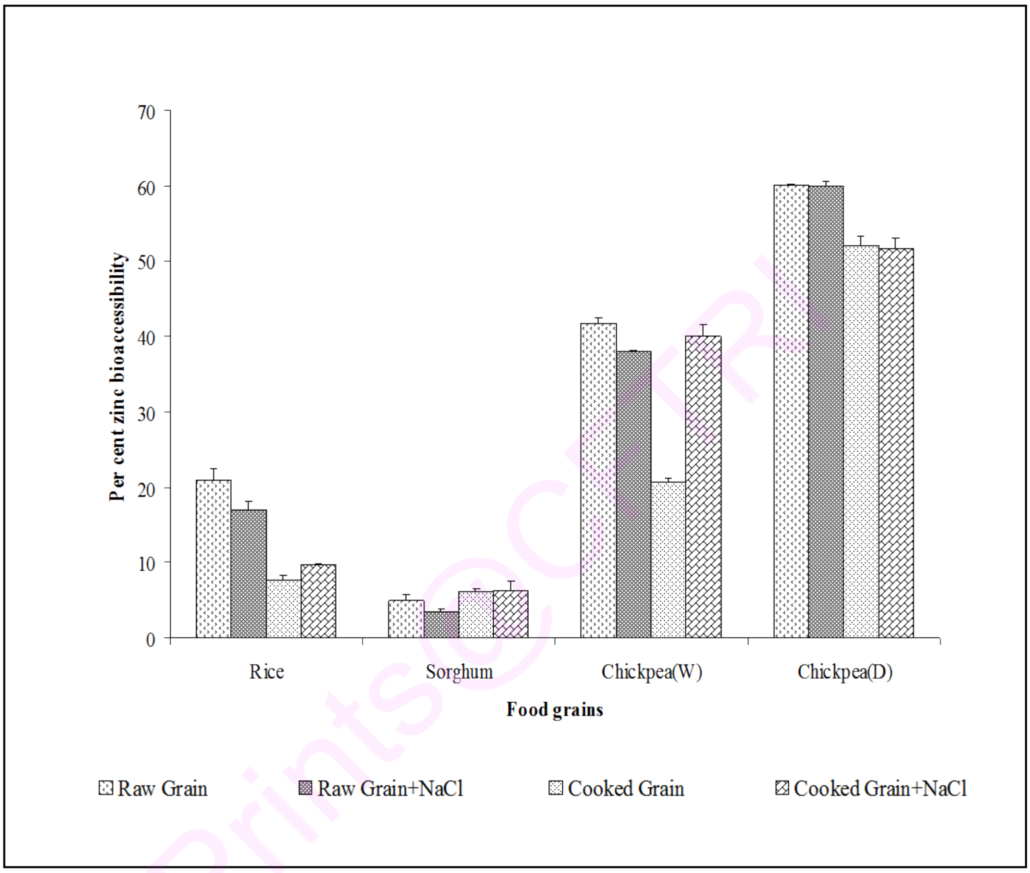


Fig.7 Zinc bioaccessibility from rice,sorghum and chickpea in the presence of exogenous common salt

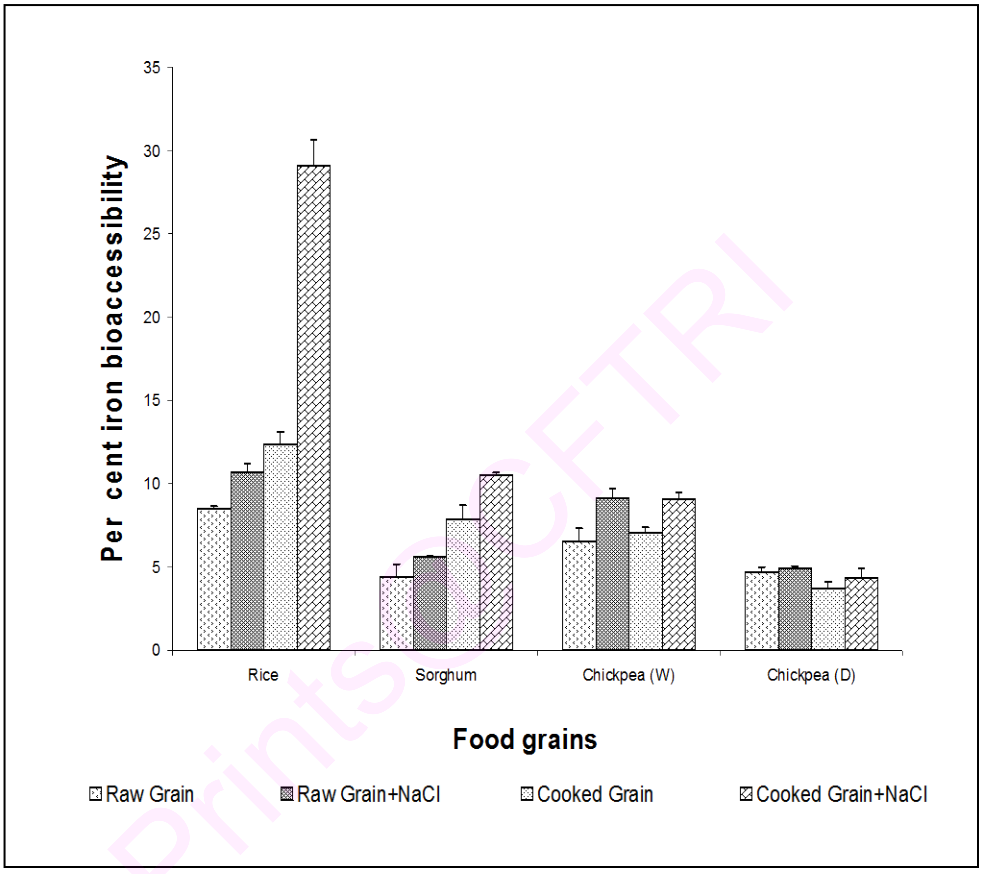


Fig.8 Iron bioaccessibility from rice, sorghum and chickpea in the presence of exogenous common salt

SUMMARY

The influence of exogenously added iron and calcium at a level up to four times the inherent concentration on the bioaccessibility of zinc from selected food grains was examined. Presence of exogenous iron at levels tested here (up to five times the intrinsic level) did not severely inhibit zinc bioaccessibility from rice and decorticated green gram. The results also suggested that the negative effect of exogenous iron is probably discernible only when zinc: iron molar ratio exceeds 1:8, as encountered during supplemental iron regimen. Exogenously added calcium did not influence the bioaccessibility of either zinc or iron from rice and wheat, even when added at levels five times those present inherently. This can be attributed to the fact that calcium *per se,* in the absence of additional phytate, may not have a negative effect on mineral availability. The influence of exogenous soy protein, on zinc and iron bioaccessibility from representative cereals- rice and sorghum was also examined. Influence of common salt along with exogenous protein on the bioaccessibility of zinc and iron from these food grains has also been specifically examined. Exogenous protein (soy protein isolate) produced contrasting effects on zinc and iron bioaccessibility from the food grains studied here. While soy protein had a negative effect on iron bioaccessibility from these food grains, the same produced an enhancing effect on zinc bioaccessibility. Exogenously added sodium chloride potentiated the positive effect of soy protein on zinc bioaccessibility, and effectively countered its negative effect on iron bioaccessibility.

CHAPTER - IV

Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains

Influence of Germination and Fermentation on Bioaccessibility of Zinc and Iron from Food Grains

INTRODUCTION

Zinc and iron bioaccessibility values in cereals and pulses commonly consumed in India in both the raw and cooked form determined by us are reported in chapter II. Bioavailability of trace minerals is known to be influenced by various dietary components that include both inhibitors and enhancers of their absorption, as well as by various food processing methods (Gibson, 1994; Sandberg, 2002). Food processing by heat generally alters the bioavailability of nutrients - both macro and micro. The digestibility and hence absorption of micronutrients such as iron is improved upon heat processing, which results in softening of the food matrix, and release of protein-bound iron, thus facilitating its absorption (Lombardi-Boccia et al, 1995). While we observed an increase in iron bioaccessibility in general from all the food grains tested here upon heat processing, the same was not true for zinc bioaccessibility (Chapter II). Bioaccessibility of zinc from food grains was observed to be lower upon heat processing, in the majority of the grains tested. Thus, heat processing of cereals and pulses produced contrasting effects on zinc and iron bioaccessibility.

Other domestic food processing methods such as germination and fermentation are known to improve mineral bioavailability by reducing the inhibitors of their absorption, such as phytic acid present in the grains (Kaur & Kawatra, 2002; Gibson & Hotz, 2001; Duhan et al, 2004). Besides reducing such factors, fermentation could also improve mineral bioavailability by virtue of the formation of organic acids, which form soluble ligands with the minerals, thereby preventing the formation of insoluble complexes with phytate. Germination and fermentation are common domestic processing methods widely used in Indian culinary. Information on the influence of germination and fermentation of cereals and legumes on the bioavailability of zinc is limiting. Combinations of cereals and pulses are fermented and made into products such as '*idli*', '*dosa*', '*dhokla*', etc., which are the primary breakfast items (Fig.1). In this investigation, we examined the influence of fermentation of combinations of rice, black gram, chickpea and green gram as in the preparation of the above fermented products, on zinc and iron bioaccessibility. Legumes such as chickpea and green gram are germinated and consumed either in the cooked or raw form as salads Fig.2). Germinated finger millet is often used in the preparation of weaning and geriatric foods. The influence of germination of the relevant grains, as commonly encountered in Indian culinary, on the bioaccessibility of zinc and iron was also examined.

MATERIALS AND METHODS

Materials:

Cereals - rice (*Oryza sativa*) and finger millet (*Eleusine coracana*), and pulses - chickpea (*Cicer arietinum*) – whole and decorticated, green gram (*Phaseolus aureus*) – whole and decorticated, and decorticated black gram (*Phaseolus mungo*), were procured locally, cleaned and used for germination and fermentation studies. Porcine pancreatin, pepsin and bile extract were from M/s Sigma Chemicals, USA. All other chemicals used here were of analytical grade. Triple distilled water was employed during the entire study. Acid-washed glassware was used throughout the study.

Total zinc and iron:

Finely ground grain samples were ashed in a muffle furnace at 550^{o}C for 5 h and dissolved in conc. HCl. Zinc and iron content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of measurements was performed using commercial standards. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron.

Bioaccessibility of zinc and iron:

Bioaccessibility of zinc and iron in various food grain samples was determined by an *in vitro* method described by Luten *et al.* (1996) involving simulated gastrointestinal digestion with suitable modifications, as described in Chapter II.

Fig.1 Photograph of fermented breakfast dishes - *'Idli'*, *'Dosa'* and *'Dhokla'*

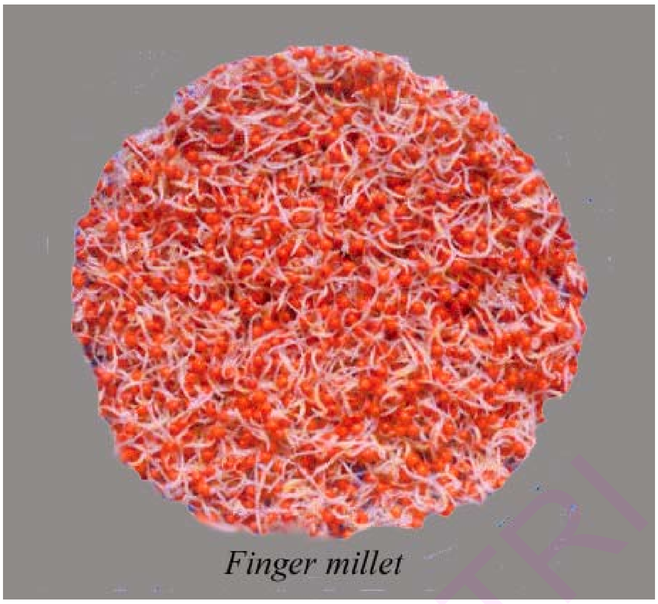


Fig.2 Photograph of germinated food grains – finger millet, green gram and chickpea

Estimation of phytate and tannin content:

Phytate in food grains was determined as phytin-phosphorous by the method of Thompson and Erdman (1982). Phytate values were computed by multiplying phytin phosphorous value by 3.55. Tannin was estimated by the modified vanillin assay of Price *et al*. (1978), using catechin as the standard.

Germination of selected grains:

Finger millet, chickpea and green gram were soaked for 16 h in triple distilled water (1: 2.5 w/v) and the soaked grains were drained of water and allowed to germinate in a BOD incubator at 25°C for 24 and 48 h.

Fermentation of cereals and legumes:

Different combinations of cereals and pulses, viz., rice + black gram (2:1) as in the preparation of *'idli'*, rice + black gram (3:1) as in the preparation of *'dosa'* and chickpea + green gram (decorticated) + black gram (decorticated) + rice (2:2:1:1) as in the preparation of *'dhokla'* were soaked in triple distilled water [grain : water = 1:2.5 w/v] for 10 h. The soaked grains were ground with the entire water to a fine batter. The batter was allowed to ferment for 14 h at laboratory temperature without addition of any exogenous starter culture. A portion of the batter was steam cooked for 10 min in the case of *'idli'* and *'dhokla'*, while a portion of the batter for *'dosa'* was pan-fried (in a non-stick pan without oil).

Determination of zinc and iron bioavailability in these variations of food samples, as well as all other chemical analyses were carried out in five replicates.

Statistical analysis:

Statistical analysis of data was done employing Student's t-test according to Snedecor and Cochran (1977).

RESULTS AND DISCUSSION

Many food items are subjected to simple household processing before they are consumed. It is possible that some of these processes may influence the availability of zinc and iron. However, no systematic study on the effect of such processes on the availability of zinc / iron in foods appears to have been carried out. This chapter reports results of a study of the effect of soaking, germination and fermentation on zinc and iron availability from selected cereals and legumes consumed in India. Among the various legumes consumed in India, green gram and chickpea are most commonly germinated prior to use in the preparation of specific traditional dishes, especially in Southern India. Germinated and malted finger millet is employed in the preparation of weaning / geriatric foods and a beverage. Hence these grains were screened in this study for evaluation of the possible influence of germination on bioaccessibility of zinc and iron. '*Idli*', '*dosa*' and '*dhokla*' are the common cereal and legume based fermented foods consumed in India. Food grains used in the preparation of these breakfast items are rice + black gram ('*idli*' and '*dosa*') and rice + black gram + chickpea + green gram ('*dhokla*') in varying proportions. These cereal-legume combinations were therefore studied here for the possible effect of fermentation on the bioaccessibility of zinc and iron.

Effect of germination:

Influence of germination of various food grains on the bioaccessibility of zinc and iron is presented in Table-1. While zinc bioaccessibility was not affected by germination of chickpea for 24 and 48 h, the same was significantly decreased in the case of finger millet (30 and 38% respectively) and green gram (38 and 44%, respectively). On the other hand, germination of these grains for 24 and 48 h significantly increased the bioaccessibility of iron by 38 and 62% (green gram), and 37 and 39% (chickpea) respectively. A 20% increase in iron bioaccessibility from finger millet was seen at the end of 48 h germination. Thus, germination of food grains had contrasting effects on the bioaccessibility of zinc and iron.

Typical food processing methods such as germination and malting have been found to enhance iron absorption due to elevated vitamin C content or reduced tannin or phytic acid content, or both (Tontisirin *et al*, 2002). These processes are known to activate phytases, which in turn hydrolyze phytate, rendering iron and zinc more available. During germination, endogenous phytase activity in cereals and legumes increases as a result of *de novo* synthesis and/or activation resulting in reductions in inositol penta- and hexa- phosphates, depending on the species and variety (Lorenz, 1980; Bartnik & Szafranska, 1987; Reddy *et al*, 1989; Chavan & Kadam, 1989). In addition, germination also reduces the content of polyphenols & tannins in some legumes (e.g. *Vicia faba*) (Camacho *et al*, 1992). Reddy *et al.* (1989) have reported reductions in phytate ranging from 36% for sprouted soya beans to 53% for germinated lentils.

Studies *in vitro* on iron bioavailability have shown a two-fold increase on germination and five to ten-fold increase on malting of minor millets (De Maeyer *et al*, 1989). Germination of pigeon pea had beneficial effect on the extractability of zinc and copper as reported by Duhan *et al.* (2004). Zinc and copper are generally present in association with phytic acid in plant foods, which may be responsible for their poor extractability. Decrease in the concentration of phytic acid by food processing such as soaking and germination, may possibly release these metallic ions in free form. Germination significantly improved zinc absorption from cooked beans (Kannan *et al*, 2001).

Chopra and Sankhala (2004) have recently examined the influence of soaking and sprouting of horse gram (*Dolichos biflorus*) and moth bean (*Phaseolus aconitifolius*), which are rich in phytate and tannin, on *in vitro* iron availability. Soaking these legumes for 8 and 16 h reduced the levels of tannins and phytates. Degradation of phytates and tannins was more pronounced after 24 h of germination. *In vitro* iron availability significantly increased after soaking as well as after germination of these legumes (Chopra & Sankhala, 2004).

Phytate content was not affected by germination of the test grains in our study, while tannin was reduced significantly, both at 24 and 48 h (Table-2). The reduction was 43 and 74% in the case of green gram, 44 and 50% in the case of finger millet and 47 and 52% in the case of chickpea, respectively after 24 and 48 h germination. Phytate and tannin are reported to be potent inhibitors of iron bioavailability (Sandberg & Svanberg, 1991; Hamdaoui *et al*, 1995). In the absence of any decrease in phytate content of the grains, reduction in tannin content during germination of the test grains in our study could be a factor that contributed to the increase in the bioaccessibility of iron. In addition, it is also possible that the vitamin C content of these grains increased during germination, and higher vitamin C contents could be an additional factor contributing to the improvement in iron bioaccessibility. Unlike iron, a relationship between tannin content of foods and zinc bioaccessibility has not been established so far. The inhibitory effect of phytate on zinc bioavailability in foods has been reported by Oberleas and Harland (1981), and Turnland *et al* (1984). The absence of any increase in zinc bioaccessibility upon germination of the tested food grains in our study is consistent with a lack of significant reduction of phytate content of these grains.

A two-fold increase in *in vitro* iron absorption has been observed in legumes and cereals germinated for different periods (Prabhavathi & Rao, 1979). Increases in iron ionizability could be observed during germination of green gram or wheat as early as 24 h, whereas such effect was seen only after extended periods (48 and 72 h) in the case of chickpea. In the case of legumes, increase was observed only with the whole grain, whereas no such changes were observed with decorticated legumes (Prabhavathi & Rao, 1979). In our study, improved iron bioacessibility was observed in chickpea germinated even for 24 h.

Thus, our observations on the improved bioaccessibility of iron upon germination of grains are in concurrence with several such observations reported in literature. The information on improved bioaccessibility of iron from finger millet upon germination reported in this study is novel. While several studies have been reported on the influence of germination of food grains on iron bioacessibility, ours is the first to report the same on zinc bioaccessibility.

Effect of soaking:

Soaking of grains is the first step in the process of germination. It is possible that soaking brings about alterations in the factors that influence mineral availability.

Soaking is claimed to reduce the phytic acid of most legumes because their phytic acid is stored in a relatively water-soluble form such as sodium or potassium phytate (Chang *et al*, 1977). Soaking may also remove polyphenols from certain beans (Ene-Obong & Obizoba, 1996). Soaking maize flour or pounded maize is reported to have resulted in 57 and 51% loss of phytate. The same authors have envisaged a reduction in phytate content and phytate: zinc molar ratio during fermentation of maize flour batter (Hotz & Gibson, 2001).

In our study, soaking of the legumes - green gram and chickpea for 16 h generally did not seem to have a beneficial influence on either zinc or iron bioaccessibility. In the case of green gram, zinc bioaccessibility was even reduced by 37% (Table-3). Our observation of reduced zinc bioaccessibility from germinated green gram (Table-1) could probably be attributed mainly to the effect of soaking itself (which is the first step in germination process) rather than that of germination. Neither soaking nor germination had any beneficial influence on the bioaccessibility of zinc from chickpea. While germination brought about a significant increase in bioaccessible iron from green gram and chickpea, the same was not observed at the stage of soaking. Hence the observed effect on iron bioaccessiblity could be attributed to changes occurring during germination. The absence of an increase in zinc and iron bioaccessibility as a result of soaking of food grains as observed by us could probably be because there was no significant reduction in phytate content of the grains as a result of 16h soaking (although phytate was not determined in soaked grains as such, germination, of which soaking is the preliminary step, did not result in any significant decrease in phytate). Soaking of grains for 16h here, did not result in the leaching of either zinc or iron. On the other hand, Lestienne *et al* (2005) found that soaking whole cereals and legumes (maize, finger millet, rice, sorghum, mung bean, cowpea and soybean) for 24h resulted in the leaching of iron, and to a lesser extent, of zinc into the soaking medium. These authors also observed significant removal of phytate as a result of soaking, and suggest that the water used for soaking, which is devoid of phytic acid, but contains leached minerals, could be used for cooking the grains, so as to conserve the leached minerals.

Table-3 Zinc and iron bioaccessibility from soaked legumes.

Food grain	Bioaccessible zinc (%)		Bioaccessible iron (%)	
	Un-processed	Soaked for 16 h	Un-processed	Soaked for 16 h
Green gram	13.06 ± 3.21	8.19 ± 0.43**	6.12 ± 1.71	6.48 ± 0.30
Chickpea	21.42 ± 1.69	17.63 ± 2.24	4.08 ± 0.61	3.57 ± 0.46

Values are mean ± SEM of 5 replicates

**Significant decrease

Effect of fermentation:

Table-4 presents the influence of fermentation on bioaccessibility of zinc and iron from combinations of cereals and legumes. The fermented batter of rice + black gram - 2:1 (‘*idli*’) and 3:1 (‘*dosa*’) showed significantly higher bioaccessibility values for zinc. The increases in zinc bioaccessibility value were as high as 71% in ‘*idli*’ and 50% in ‘*dosa*’, respectively. Such an increased bioaccessibility of zinc however, was not evident in the case of fermented ‘*dhokla*’ batter; instead there was a slight decrease (10%). Since these fermented batters are heat-processed in the preparation of respective dishes, bioaccessibility of these minerals was also determined in the final products. In the case of ‘*idli*’, the zinc bioaccessibility value, although higher (27%) than that of unprocessed grains, was lower (26%) than that of the uncooked fermented batter. In the case of ‘*dosa*’, the zinc bioaccessibility value similarly decreased upon heat processing of the fermented batter (46% lower), and this value was 18% lower than that of the unprocessed grains. In the case of ‘*dhokla*’, the zinc bioaccessibility value decreased by 23% upon heat processing of the fermented batter, this final value being 31% lower than the unprocessed grain-combination.

Fermentation of the combination of rice and black gram at both the proportions examined enormously improved the bioaccessibility of iron. Bioaccessible iron in the fermented ‘*idli’* batter was 276% higher and that in the *dosa* batter was 127% higher compared to the untreated grains used here. Heat treatment of the batter further increased the bioaccessibility of iron by 92% in the case of ‘*dosa*’, while that in the ‘*idli*’ batter did not change. Thus, fermentation and heat processing as in the preparation of ‘*dosa*’ resulted in a net increase of 335% in the bioaccessibility of iron from rice-black gram combination. Fermentation of a combination of chickpea, green gram, black gram and rice as in the preparation of ‘*dhokla*’, however did not improve the bioaccessible iron content of the native grains. Heat processing of the fermented batter of ‘*dhokla’* also did not alter the bioaccessible iron content; thus, the bioaccessible iron value of ‘*dhokla’* remained the same as that of the native grain combination used.

Microbial fermentation can enhance iron and zinc bioavailability via hydrolysis of phytate by microbial phytase derived from naturally occurring microflora on the surface of the cereal grains (Sandberg, 1991). The beneficial effect of fermentation on mineral bioavailability could also be attributed to the formation of organic acids during this process, which form soluble ligands with iron and zinc (Tontisirin *et al*, 2002). In fact, we did evidence a reduction in the pH by 1.5 units as a result of fermentation of the batter of '*idli*', '*dosa*' and '*dhokla*' in our study. This reduction in pH is certainly due to the synthesis of organic acids during fermentation. In Latin American countries, household food processing methods such as fermentation and germination are used in formulating infant foods (Devadas, 1998). *In vitro* measurements of soluble iron have been reported in cereal porridges prepared with soaking and fermenting flour slurries (Svanberg *et al*, 1993). Greater femoral zinc in rats fed with diets containing fermented soybean meal as compared to regular soybean meal has been reported, which is probably resulting from increased zinc solubility in the small intestine (Hirabayashi *et al*, 1998).

Besides improving organoleptic properties, soaking and fermentation of cereal flours activate phytases, which in turn hydrolyze phytate, improving iron and zinc bioavailability (Svanberg *et al*, 1993).

Reductions in phytate content have been reported during lactic fermentation in the case of maize flour (Amoa & Muller, 1976) and leavening of wheat flour dough (Tangkongchitr *et al*, 1982). An increase in *in vitro* iron bioavailability from iron fortified dairy products (acidified milk and yoghurt) has been evidenced following milk fermentation or acidification. Lactic acidification & fermentation also increased zinc availability *in vitro* (Drago & Valencia, 2002). In our investigation, natural fermentation of cereal-legume combinations significantly reduced both phytate and tannin (Table-5) from the test grains. Phytate was completely removed during fermentation of the '*idli*' batter, while the reduction was 50 and 28% during fermentation of '*dosa*' and '*dhokla*' batter, respectively. Only traces of tannin were detectable in the fermented batters of '*idli*', '*dosa*' and '*dhokla*'. Absence of any positive influence of fermentation on mineral bioaccessibility in the case of '*dhokla*' batter could be attributed to the continued presence of significant amounts of phytate. The additional legumes - chickpea and green gram present in '*dhokla*' apart from rice and black gram (constituent grains for '*idli*' and '*dosa*') have contributed to this significant phytate content.

In one of the earliest studies on the influence of food processing on *in vitro* iron availability, Prabhavati and Rao (1979) have reported that fermentation of rice and black gram mixture and subsequent cooking (as in the preparation of '*idli*') did not result in any change in the ionizable iron content. Our present observation on the fermented food grains differs from this report. Most of these earlier studies reported on the influence of fermentation on mineral bioavailability involved the use of externally added starter cultures for fermentation, while our study reports the influence of fermentation by cultures of lactic acid bacteria naturally associated with the grain. While several studies have been reported on the influence of lactic fermentation (using starter cultures) of grains flours on mineral bioacessibility, information on influence of natural fermentation, as encountered at the household level on zinc bioaccessibility is limited.

Thus, Soaking, germination and fermentation of cereals & legumes (in which phytate is in the cotyledons) offers a practical household method to reduce inhibitors of mineral absorption especially phytic acid and tannin, thereby contributing to enhanced zinc and iron absorption. In fact, such processing of cereals and legumes as part of the daily culinary are in wide practice across the Indian subcontinent.

SUMMARY

The present study has revealed that while germination of green gram, chickpea and finger millet for 24 and 48 h significantly enhanced the bioaccessibility of iron, bioaccessibility of zinc was not beneficially affected. In the absence of any decrease in phytate content of the grains, reduction in tannin content during germination of the test grains could have contributed to the increase in the bioaccessibility of iron. Fermentation of the batter of cereal-pulse combination as in the preparation of 'i*dli*' and 'd*osa*' significantly enhanced the bioaccessibility of both zinc and iron, the extent of increase in the case of iron being still better. However, such beneficial influence of fermentation on zinc and iron bioaccessibility was not observed in the case of the cereal-pulse combination used for the preparation of '*dhokla*'. Significant reduction of both phytate and tannin during fermentation of cereal-legume combinations of the '*idli'* and 'd*osa'* batter must have contributed to the observed increase in mineral

bioaccessibility. Absence of any positive influence of fermentation on mineral bioaccessibility in the case of ‘d*hokla’* batter could be attributed to the continued presence of significant amounts of phytate or any other protein that binds these micronutrients contributed by additional legumes - chickpea and green gram present in ‘d*hokla’*. Thus, Soaking, germination and fermentation of cereals & legumes (in which phytate is in the cotyledons) offers a practical household method to reduce inhibitors of mineral absorption especially phytic acid and tannin, thereby contributing to enhanced zinc and iron absorption. In fact, such processing of cereals and legumes as part of the daily culinary is in wide practice across the Indian subcontinent.

Table-1 Influence of germination on bioaccessibility of zinc and iron from finger millet, green gram and chickpea.

Food grain	Bioaccessible zinc (%)			Bioaccessible iron (%)		
	Native	Germinated – 24 h	Germinated – 48 h	Native	Germinated – 24 h	Germinated – 48 h
Finger millet	3.94 ± 0.27	2.77 ± 0.43**	2.44 ± 0.13**	24.55 ± 0.38	26.29 ± 0.70	29.54 ± 0.28*
Green gram	37.47 ± 4.78	23.24 ± 2.48**	21.01 ± 1.60**	5.05 ± 0.10	6.95 ± 0.50*	8.18 ± 0.09*
Chickpea	45.09 ± 1.76	40.47 ± 2.89	40.13 ± 1.58	6.39 ± 0.31	8.78 ± 0.40*	8.87 ± 0.26*

Values are mean ± SEM of 5 replicates

*Significant increase; **Significant decrease

Table-2 Tannin and phytate content of germinated finger millet, green gram and chickpea.

Food grain	Tannin			Phytate		
	Native	Germinated – 24 h	Germinated – 48 h	Native	Germinated – 24 h	Germinated – 48 h
Finger millet	973.9 ± 10.3	547.2 ± 6.43**	482.6 ± 2.16**	215.4 ± 5.62	203.9 ± 4.78	204.6 ± 3.28
Green gram	450.0 ± 4.78	258.7 ± 3.66**	117.4 ± 1.80**	219.0 ± 6.00	208.9 ± 3.52	189.5 ± 8.30
Chickpea	215.2 ± 2.76	115.0 ± 2.29**	104.3 ± 1.42**	180.8 ± 2.81	170.0 ± 2.45	164.2 ± 5.22

Values expressed as mg per 100 g are mean ± SEM of 5 replicates

**Significant decrease compared to unprocessed sample

Table-4 Influence of fermentation on bioaccessibility of zinc and iron from selected combinations of cereals and pulses

Food grain	Bioaccessible zinc (%)			Bioaccessible iron (%)		
	Raw grains	Fermented Batter - Raw	Fermented batter - Cooked	Raw grains	Fermented Batter - Raw	Fermented batter - Cooked
Idli:						
Rice : Black gram (D) (2:1)	31.73 ± 1.42	54.29 ± 5.92*	40.29 ± 2.50*	6.50 ± 0.63	24.50 ± 0.31*	23.05 ± 1.59*
Dosa:						
Rice : Black gram (D) (3:1)	29.66 ± 1.90	44.59 ± 3.66*	24.29 ± 1.36	6.90 ± 0.67	15.68 ± 1.88*	30.06 ± 3.10*
Dhokla:						
Chickpea : Green gram (D) : Black gram (D) : Rice (2:2:1:1)	53.99 ± 2.91	48.34 ± 3.39	37.10 ± 6.13**	11.96 ± 0.57	13.37 ± 0.55	13.34 ± 1.04

Values are mean ± SEM of 5 replicates

D: Decorticated

*Significant increase compared to raw sample; **Significant decrease compared to raw sample

Table-5 Tannin and phytate content of fermented cereal-pulse combinations

Food grain	Tannin		Phytate	
	Raw grains	Fermented Batter - Raw	Raw grains	Fermented Batter - Raw
Idli: Rice : Black gram (D) (2:1)	13.04 ± 0.64	Traces**	85.9 ± 2.15	Traces**
Dosa: Rice : Black gram (D) (3:1)	9.50 ± 0.35	Traces**	65.4 ± 1.80	32.7 ± 2.55**
Dhokla: Chickpea : Green gram (D): Black gram (D) : Rice (2:2:1:1)	182.6 ± 2.41	Traces**	193.3 ± 3.16	139.1 ± 4.50**

Values are mean ± SEM of 5 replicates

D: Decorticated

**Significant decrease compared to raw sample

CHAPTER - V

Influence of food acidulants on bioaccessibility of zinc and iron from selected food grains

Influence of Food Acidulants on Bioaccessibility of Zinc and Iron from Selected Food Grains

INTRODUCTION

Bioavailability of micronutrients, particularly zinc and iron is low from plant foods (Gibson, 1994; Sandberg, 2002). Bioavailability of trace minerals such as iron is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption. Although not exhaustively evidenced, it is possible that the bioavailability of zinc from food grains is similarly influenced by such diverse factors coexistent in them. Organic acids are known to promote the absorption of iron from plant foods (Gillooly *et al*, 1983). Acidulants such as lime, tamarind, kokum and amchur are commonly used in Indian culinary to impart a desirable sour taste to certain food preparations. In the absence of any information on the influence of food acidulants on the bioaccessability of zinc from food grains, it would be relevant to examine the same. The present investigation was undertaken to study the influence of common food acidulants namely citric acid (acid constituent of lime), tamarind (*Tamarindus indica*), amchur (*Mangifera indica*) and kokum (*Garcinia indica*) on the bioaccessibility of zinc from selected food grains, both in the raw and cooked forms. The comparative influence of the same acidulants on iron bioaccessibility from these food grains was evaluated.

The earlier chapters have dealt with zinc and iron bioaccessibility values from individual food grains (one at a time) - both raw and heat- processed, from particular germinated grains, from fermented combinations of cereal and pulse of a specific proportion. The presence of exogenous common salt (Chapter-III) and of a particular acidulant (the present chapter) have also been examined for their influence on zinc and iron bioaccessibility from selected food grains. A study of the bioaccessibility of minerals from whole meals, the actual form in which staple cereals and pulses are consumed, would be the most practical assessment of zinc and iron bioaccessibility from the food grains from which they are derived. Therefore, we have also examined zinc and iron bioaccessibility from representative heat- processed whole meals which consist of a staple cereal and pulses, with a variety of other ingredients such as common salt, acidulants, vegetables, fat, and spices. For this purpose, four meals based on rice, finger millet, wheat and sorghum, the staple cereals commonly consumed by populations across India were specifically examined for zinc and iron bioaccessibility.

MATERIALS AND METHODS

Materials:

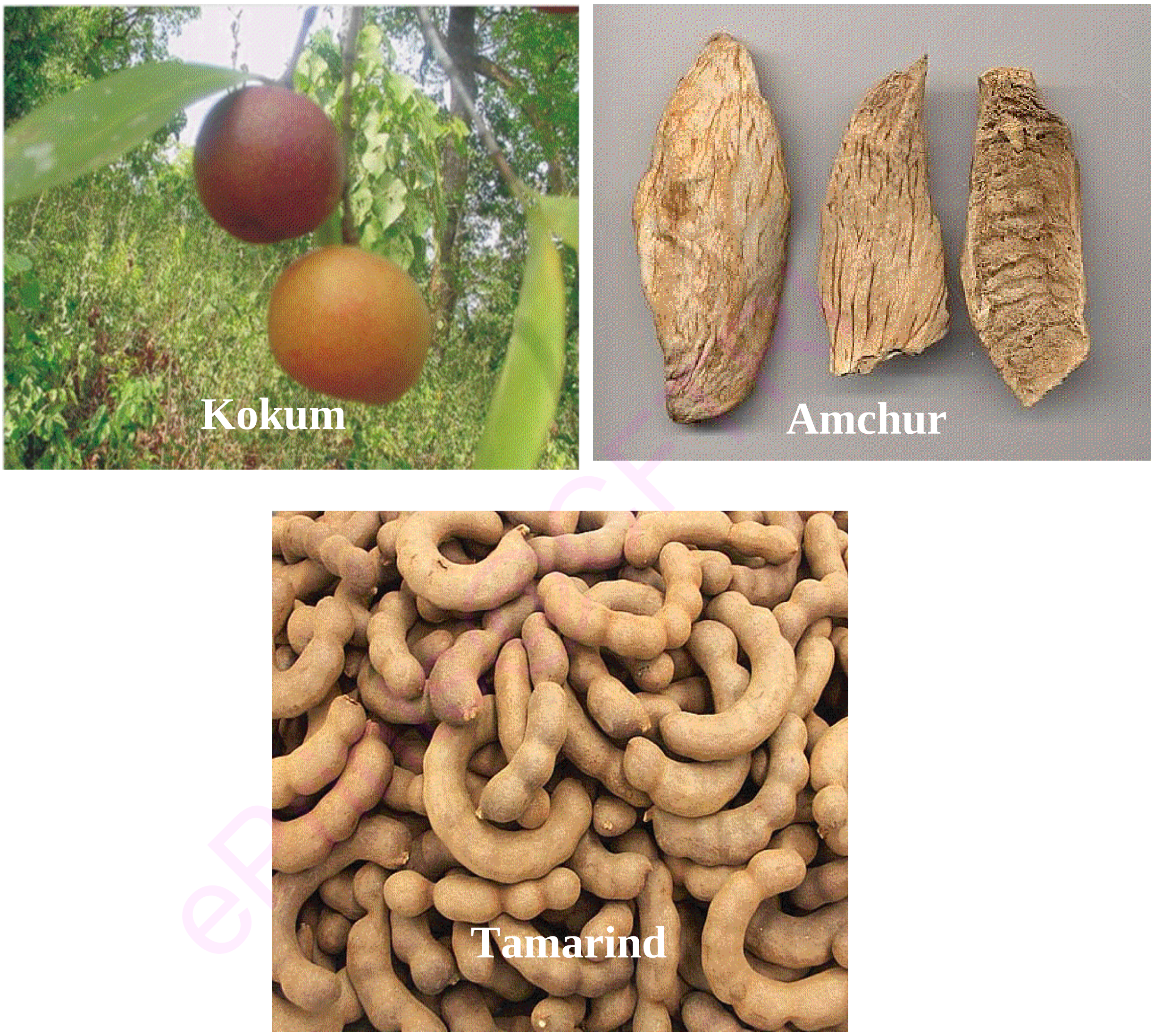
The cereal rice (O*ryza sativa*) and legumes Chickpea (*Cicer arietinum)* – whole and decorticated, green gram (*Phaseolus aureus*) – decorticated, and red gram (Cajanus *cajan*), decorticated, were procured locally, cleaned and used for bioaccessibility studies. Food acidulants, which were examined in this study, namely, tamarind (*Tamarindus indica*) powder, amchur (dried raw mango - *Mangifera indica*) and kokum (*Garcinia indica*) were also procured locally. The citric acid used was of analytical grade.

For the studies with whole meals, staple cereals- rice, wheat, finger millet and sorghum and pulses- red gram, green gram (d) and French bean, fresh vegetables, dry tamarind powder, sun flower oil, spice mix (sambar powder) and other spices were procured locally.

Pepsin (from porcine pancreas), bile extract (porcine) and pancreatin (porcine) were procured from Sigma Chemical Company, USA. All other chemicals used were of analytical grade. Triple distilled water was employed throughout the study. Acid–washed glassware was used during the entire study.

Total zinc and iron:

Food samples were ground finely and ashed in a muffle furnace at 550^{o}C for 5 h and the ash was dissolved in conc. HCl. Zinc and iron content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of measurements was performed using commercial standards. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron.



Photographs of food acidulants examined in this study

Effect of acidulants on bioaccessibility of zinc and iron:

Bioaccessibility of zinc and iron was determined in selected food grains (rice, chickpea, red gram and green gram) in the absence and presence of individual acidulants as listed. The acidulants were included at levels that produced a decrease in pH by 1 unit.

1. Food grain (10 g) + No acidulant
2. Food grain (10 g) + Citric acid (0.05 g)
3. Food grain (10 g) + Tamarind powder (0.75 g)
4. Food grain (10 g) + Amchur powder (0.75 g)
5. Food grain (10 g) + Kokum powder (0.75 g)

Two parallel sets of above samples were examined for mineral bioaccessibility in raw and cooked forms. Determination of zinc and iron bioavailability in these variations of food samples, as well as all other chemical analyses were carried out in five replicates.

Bioaccessibility of zinc and iron in various food grain samples was determined by an *in vitro* method described by Luten *et al.* (1996) involving simulated gastrointestinal digestion with suitable modifications, as described in chapter II.

Heat processing of food grains:

To examine the influence of acidulants on zinc and iron bioaccessibility from heat – processed food grains, the selected food grains (10 g) were pressure-cooked in 30 ml of triple distilled water for 10 min (15 psi), in the presence of the acidulants. The cooked samples were homogenized and used for the determination of mineral bioaccessibility as above.

Estimation of tannin:

Tannin was estimated by the modified vanillin assay of Price *et al*. (1978), using catechin as the standard.

***Preparation of representative meals*:**

The four representative meals, whose composition is given in Tables 1-4, were prepared as commonly practiced in Indian households. The individual items of these

Table-1 Composition of rice-based meal

Meal Item	Ingredients	Amount (g)
Rice	Milled polished rice	75
Sambar	Onion	25
	Potato	25
	Tomato	25
	Sambar powder	10
	Salt	5
	Oil	2
	Tamarind powder	2
	Turmeric	1
	Mustard	1
Curry	Amaranth leaves	25
	Oil	5
	Salt	2.5
	Chilli (Dry)	2.5
	Mustard	1
Curds	Curds	50 (ml)

Table-2 Composition of wheat-based meal

Meal Item	Ingredients	Amount (g)
Chapathi	Wheat flour	75
	Oil	20 (ml)
	Salt	2.5
Curry	French bean	10
	Potato	75
	Fenugreek leaves	25
	Oil	10
	Salt	5
	Chilli (dry)	2.5
Curds	Curds	50

Table-3 Composition of finger millet-based meal

Meal Item	Ingredients	Amount (g)
Ragi ball	Ragi flour	75
Sambar	Red gram dhal	10
	Fenugreek leaves	25
	Sambar powder	10
	Salt	5
	Oil	2.5
	Tamarind	2.0
	Mustard	1
Salad	Carrot	25
	Cucumber	25
	Tomato	25
Curds	Curds	50 (ml)

Table-4 Composition of sorghum- based meal

Meal Item	Ingredients	Amount (g)
Roti	Sorghum flour	75
	Salt	2.5
	Oil	20
Dhal	Green gram (decorticated)	10
	Onion	35
	Tomato	40
	Amaranth leaves	25
	Salt	5
	Oil	10
	Chilli	2
	Curds	50(ml)

four meals were cooked separately and each meal was homogenized in a stainless steel blender before analysis. Zinc and iron bioaccessibility from these meals was determined as described in Chapter - II.

Statistical analysis:

Statistical analysis of analytical data was done employing Student's t-test according to Snedecor and Cochran (1977).

RESULTS AND DISCUSSION

Organic acids such as citric, malic, tartaric, and ascorbic acid are well documented to have a significant enhancing influence on iron bioavailability (Gillooly *et al*, 1983). Information on such possible influence of organic acids on the bioavailability of zinc is, however, lacking. Several acidulants are commonly employed in Indian dietary, which, being a source of one or the other of organic acids, could possibly alter the bioavailability of the trace mineral zinc from food sources. The present study, which has examined the bioaccessibility values of zinc and iron from selected food grains both in presence and absence of individual acidulants, has evidenced differences, which provide an insight into the contribution of the acidulant component to the availability of trace minerals.

Influence of citric acid on zinc and iron dialyzability:

Influence of citric acid on bioaccessibility of zinc from rice, chickpea, green gram and red gram is shown in Fig.1. Presence of citric acid considerably increased the bioaccessibility of zinc from rice and chickpea, to an extent of 44 and 40% from raw and cooked rice, and to an extent of 29 and 31 % from raw and cooked chickpea, respectively. The zinc bioaccessibility from cooked red gram was enhanced by an extent of 11%. While citric acid did not have a similar positive influence on zinc bioaccessibility from the other food grains examined, it negatively influenced the same from raw decorticated chickpea (23 % decrease).

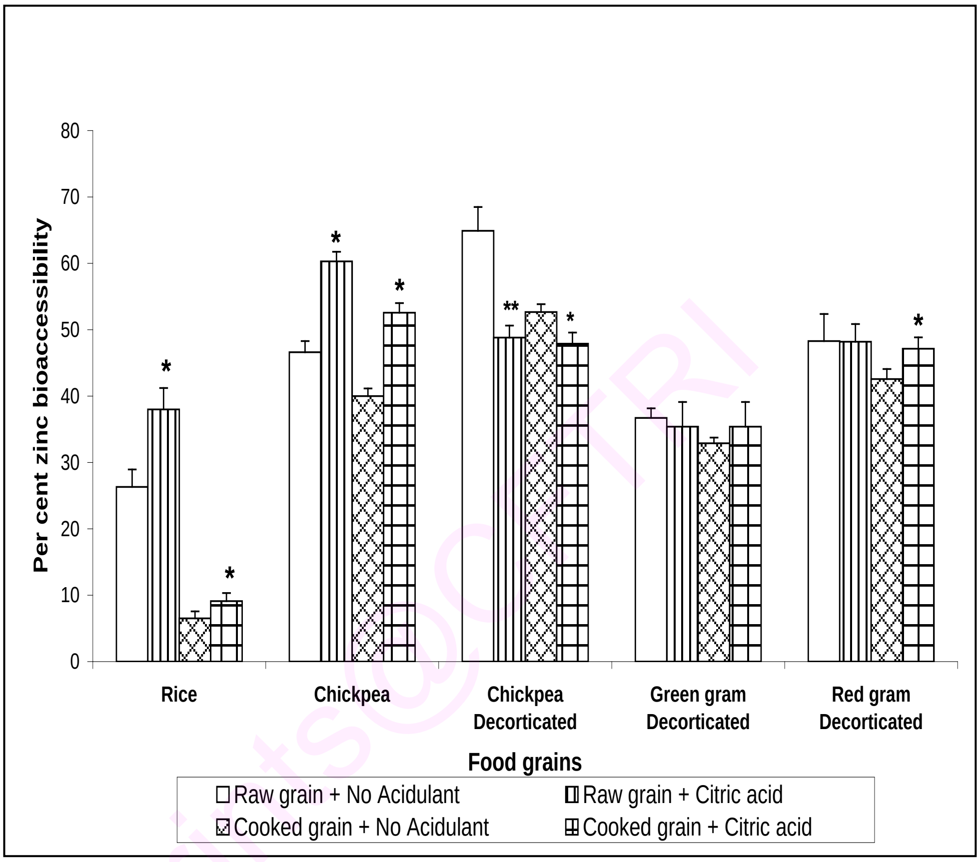


Fig.1 Effect of citric acid on zinc bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

Influence of citric acid on bioaccessibility of iron from food grains is presented in Fig. 2. Citric acid generally enhanced iron bioaccessibility from the tested food grains. The increase in bioaccessibility was 30 and 62 % from raw and cooked rice; around 62 % from both raw and cooked decorticated chickpea; 22 and 31 % from raw and cooked decorticated green gram. The increase in iron bioaccessibility in presence of citric acid was 86 and 55 % from cooked decorticated red gram and whole chickpea, respectively, while this acidulant had no effect on iron bioaccessibility from the two raw grains. Thus, the beneficial influence of citric acid on iron bioaccessibility was relatively more when the grains were cooked along with the acidulant. This trend, however, was not evident in the case of zinc bioaccessibility. Citric acid, the acidic constituent of lime, has been employed in this study to represent the parent acidulant, for the sake of convenience.

Influence of amchur on zinc and iron dialyzability:

Bioaccessibility of zinc from rice, chickpea, green gram and red gram in presence of the acidulant - amchur is shown in Fig. 3. Amchur enhanced the bioaccessibility of zinc from all the food grains examined. The increase in zinc bioaccessibility from rice was more than 100 % from raw and cooked grain. Zinc bioacessibility from chickpea (whole grain) was increased by 27 (raw grain) and 22 % (cooked grain), while zinc bioaccessibility from decorticated chickpea was increased by 23 and 11 % in the raw and cooked grains, respectively. An increase in zinc bioaccessibility of 59 % was observed from raw decorticated green gram. No influence of amchur on zinc bioaccessibility was evident in red gram, either raw or cooked, and in cooked green gram.

Fig. 4 presents the iron bioaccessibility from food grains as influenced by the acidulant amchur. Amchur had an enhancing influence on iron bioaccessibility generally from the raw food grains. It increased the bioaccessibility value by 11, 37, and 350% in rice, decorticated green gram and decorticated red gram, respectively. On the other hand, iron bioaccessibility from raw chickpea was decreased by 41%. Amchur significantly enhanced the bioaccessibility from cooked chickpea (as much as 114%), while it generally had a negative influence on iron bioaccessibility from the other cooked grains.

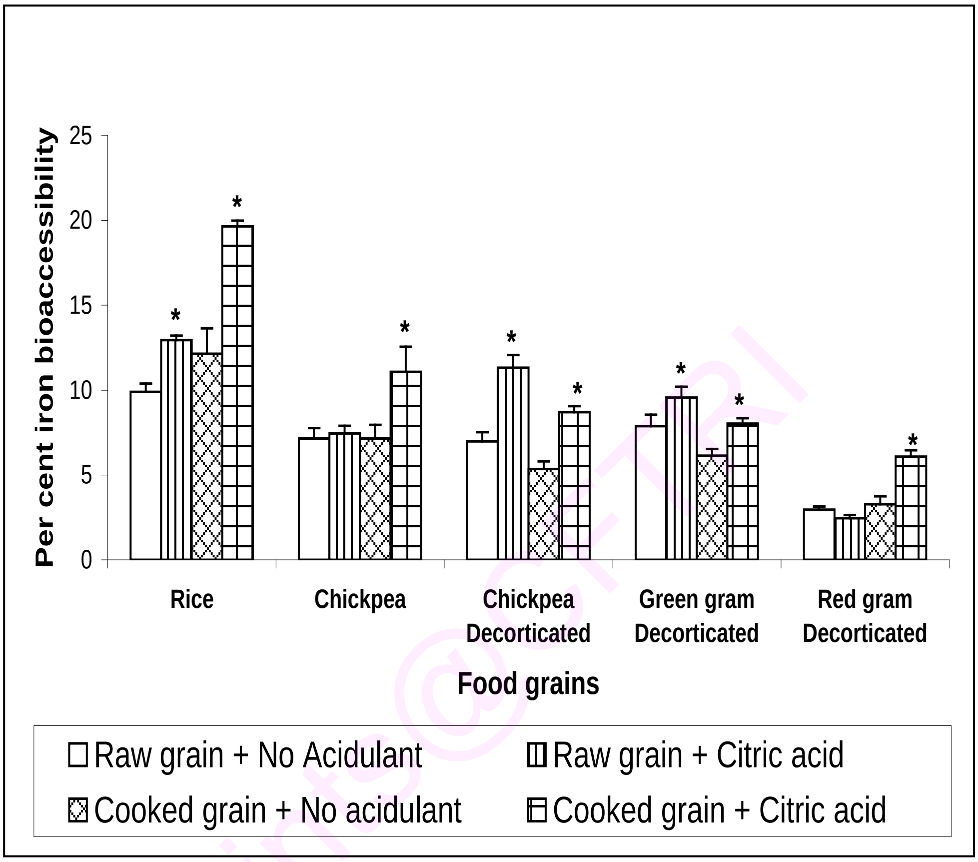


Fig.2 Effect of citric acid on iron bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

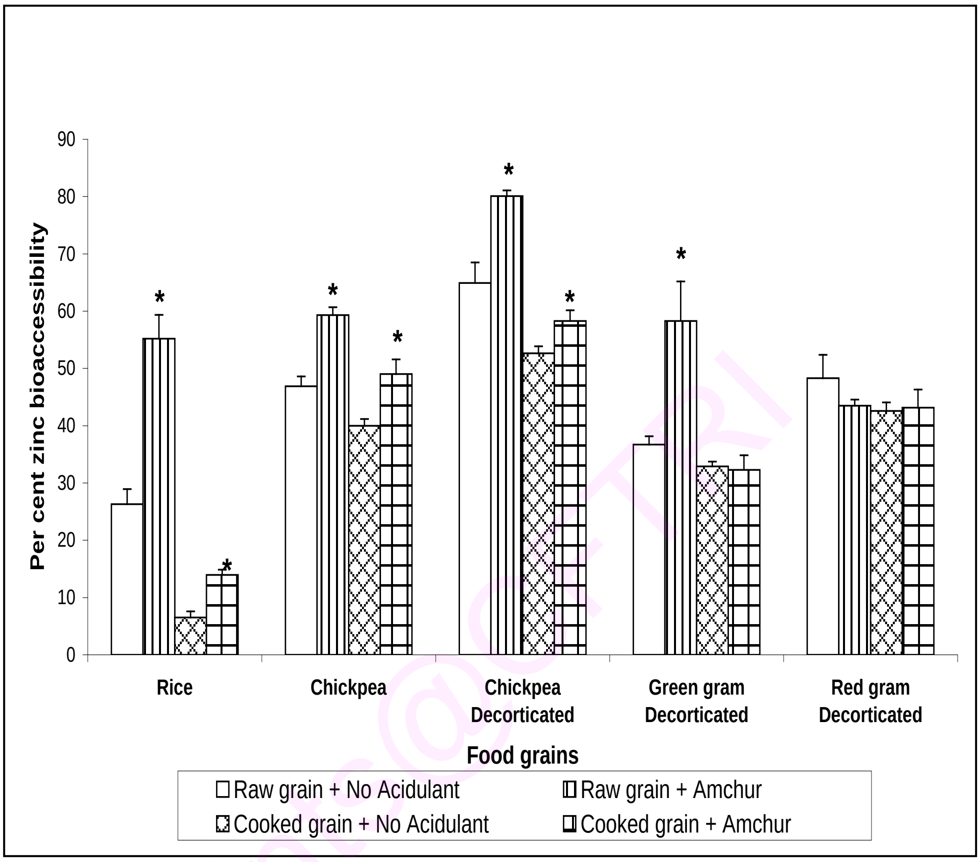


Fig.3 Effect of amchur on zinc bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

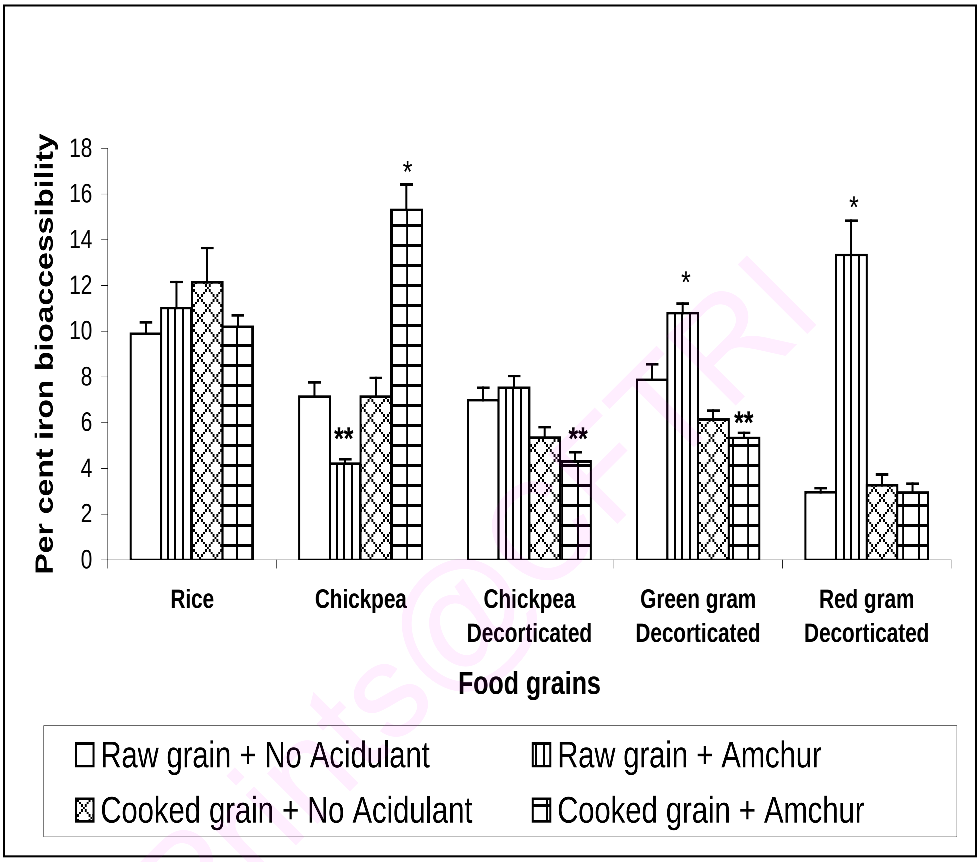


Fig.4 Effect of amchur on iron bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

Influence of tamarind on zinc and iron dialyzability:

The effect of tamarind on the bioaccessibility of zinc from food grains is shown in Fig. 5. Inclusion of tamarind produced an increase of 18% in the bioaccessible zinc concentration of raw chickpea. In all other cases, tamarind did not have any effect, or even produced a negative effect (a decrease by 10 to 57%). Whereas the negative influence of tamarind was seen in cooked rice (57% decrease) with no effect in the raw grain, the opposite trend was observed in the case of decorticated chickpea, where a decrease by 32% was observed in the raw grain.

Fig. 6 presents the influence of tamarind on the bioaccessibility of iron from food grains. Tamarind similarly showed a negative influence on iron bioaccessibility from all the food grains examined, and this ranged from a decrease by 28% in raw chickpea to 58% in cooked decorticated chickpea. The only exception to this was in the case of cooked whole chickpea, where tamarind enhanced the iron bioaccessibility (by 22%).

Influence of kokum on the dialyzability of zinc and iron:

The effect of kokum on the bioaccessibility of zinc from food grains is shown in Fig. 7. Kokum generally had a decreasing influence on the bioaccessibility of zinc from the food grains studied, except in the case of whole chickpea, where it produced an increase by 18% in the cooked grain. The decrease in bioaccessibility value produced by kokum ranged from 10% in raw decorticated chickpea to 39% in cooked rice. Influence of kokum on the bioaccessibility of iron from food grains is presented in Fig. 8. Kokum had a negative influence on the bioaccessibility of iron from all the food grains tested, this effect being as high as 88% in cooked decorticated green gram. As in the case of tamarind, the only exception was cooked whole chickpea, where an increase in iron bioaccessibility (by 60%) was observed. Thus, among the four common food acidulants examined, amchur and citric acid exerted a positive influence on mineral bioaccessibility in more number of samples. The observation that citric acid and amchur had the similar effect of enhancing the bioaccessibility of zinc could be due to the fact that citric acid is the main acidulant in amchur too, with minor amounts of malic acid. Even though the other two acidulants – tamarind and kokum have been included in amounts that produce the same level of acidity as amchur and citric acid, they did not exhibit a similar positive effect on mineral availability.

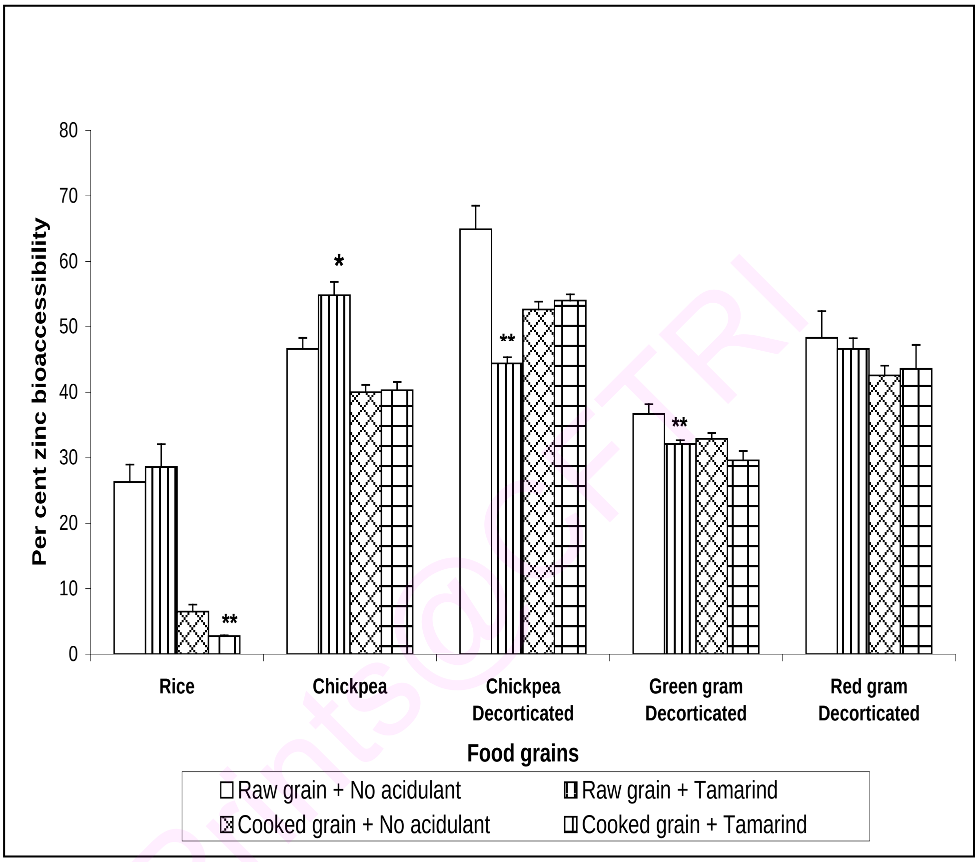


Fig.5 Effect of tamarind on zinc bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

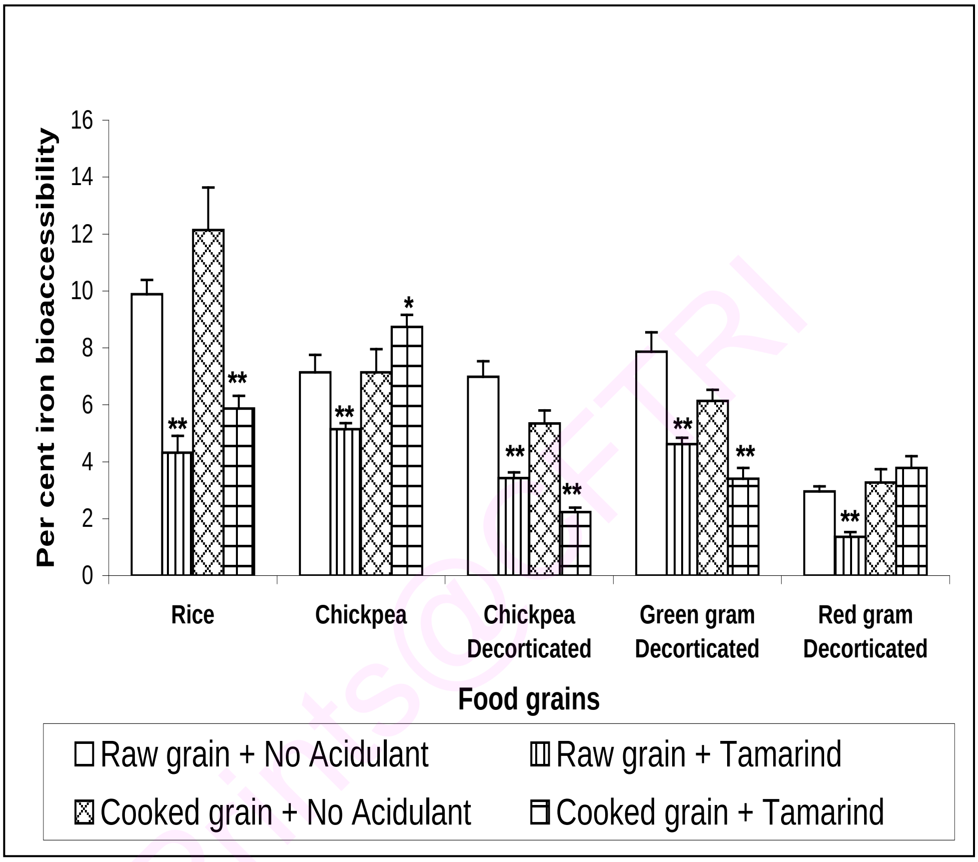


Fig.6 Effect of tamarind on iron bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

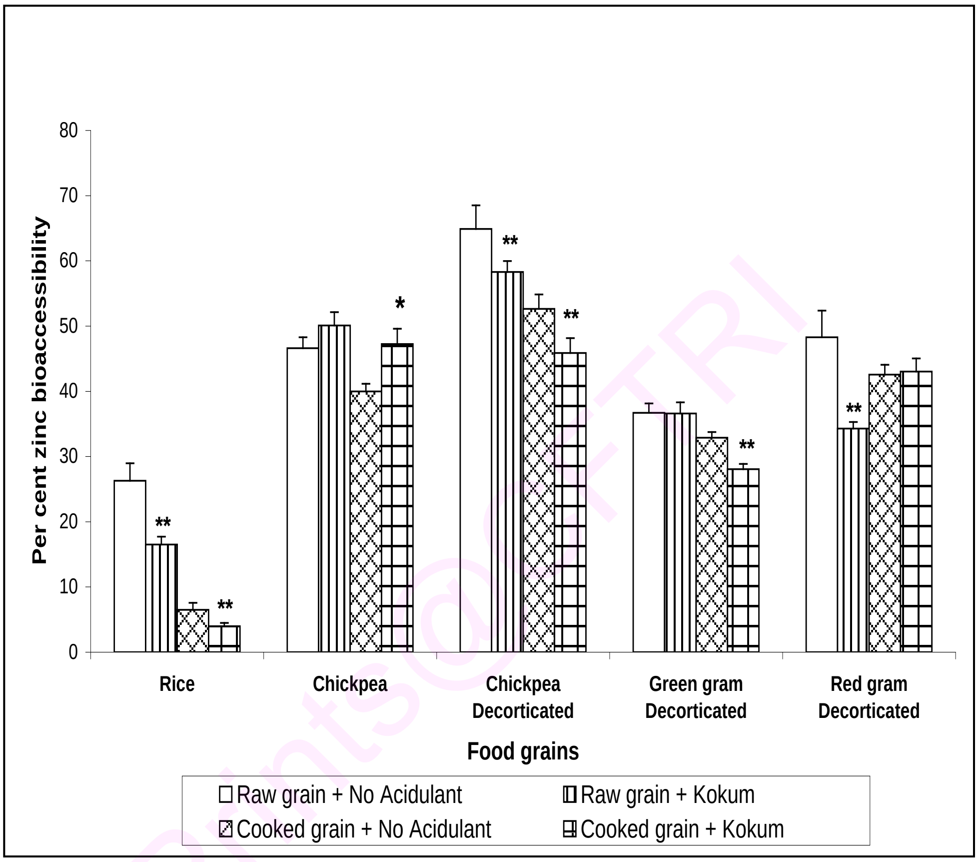


Fig.7 Effect of kokum on zinc bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

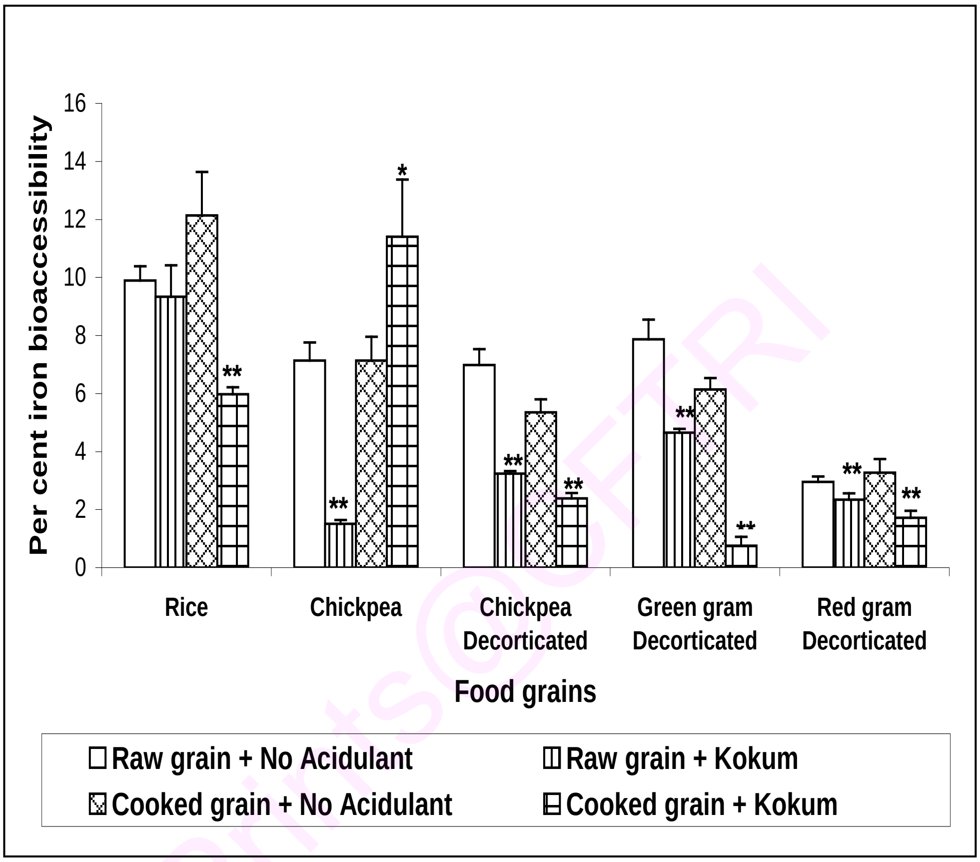


Fig.8 Effect of kokum on iron bioaccessibility from food grains.

Values represent mean ± SEM of 5 independent determinations
* Significant increase; ** Significant decrease

Tamarind has tartaric acid as the main acid, while kokum has hydroxycitric acid and minor quantities of tartaric acid. Thus, among various organic acids, citric acid has the maximum beneficial effect with regard to enhancing the mineral bioaccessibility. This absence of a comparable positive influence of the other two acidulants – tamarind and kokum may also be attributable to the high concentrations of tannin present in them (Fig.9). These two acidulants have contributed significant amounts to the total tannin content of the food samples examined. Tannin is known to strongly inhibit mineral absorption (O'Dell, 1984). Although there are reports that organic acids (citric, malic, ascorbic and tartaric) enhance the absorption of iron from foods, the commonly used food acidulants have not been examined in this context. The present study is the first report on the influence of amchur, tamarind and kokum (as sources of organic acids) on mineral bioaccessibility.

The beneficial influence of citric acid on zinc bioaccessibility observed here roughly correlated with the concentration of phytic acid inherent in the food grain (Chapter II). Thus, pronounced enhancement in the zinc bioaccessibility value was evident in rice and chickpea, which had lower concentrations of phytic acid (150 and 263 mg/100g, respectively) compared to the other grains studied (324 mg/100g in decorticated chickpea to 630 mg/100g in decorticated green gram). Phytate is known to be a potent inhibitor of trace element absorption (Lonnerdal, 2002). Citric acid was thus unable to counter the inhibitory effect of phytate on zinc bioacessibility, where the concentrations of the latter in the grains were higher. However, amchur significantly enhanced zinc dialyzability even from those food grains that had higher concentration of phytate. Such an inverse relationship between the beneficial influence of acidulant and inherent phytate concentration was not evident in the case iron bioaccessibility. Citric acid enhanced the bioaccessibility of iron from all the grains tested, irrespective of their phytate concentration. We have earlier documented that iron bioaccessibility from food grains, especially pulses, was not negatively influenced by the inherent phytate (Chapter - II). Citric acid (of lime) and amchur have been evidenced here to significantly enhance the dialyzability of both zinc and iron from rice. The usage of lime and raw mango in combination with rice in preparations such as "lemon rice" and "mango rice" is actually in vogue in Southern India. Such a practice could thus prove advantageous in terms of zinc absorption.

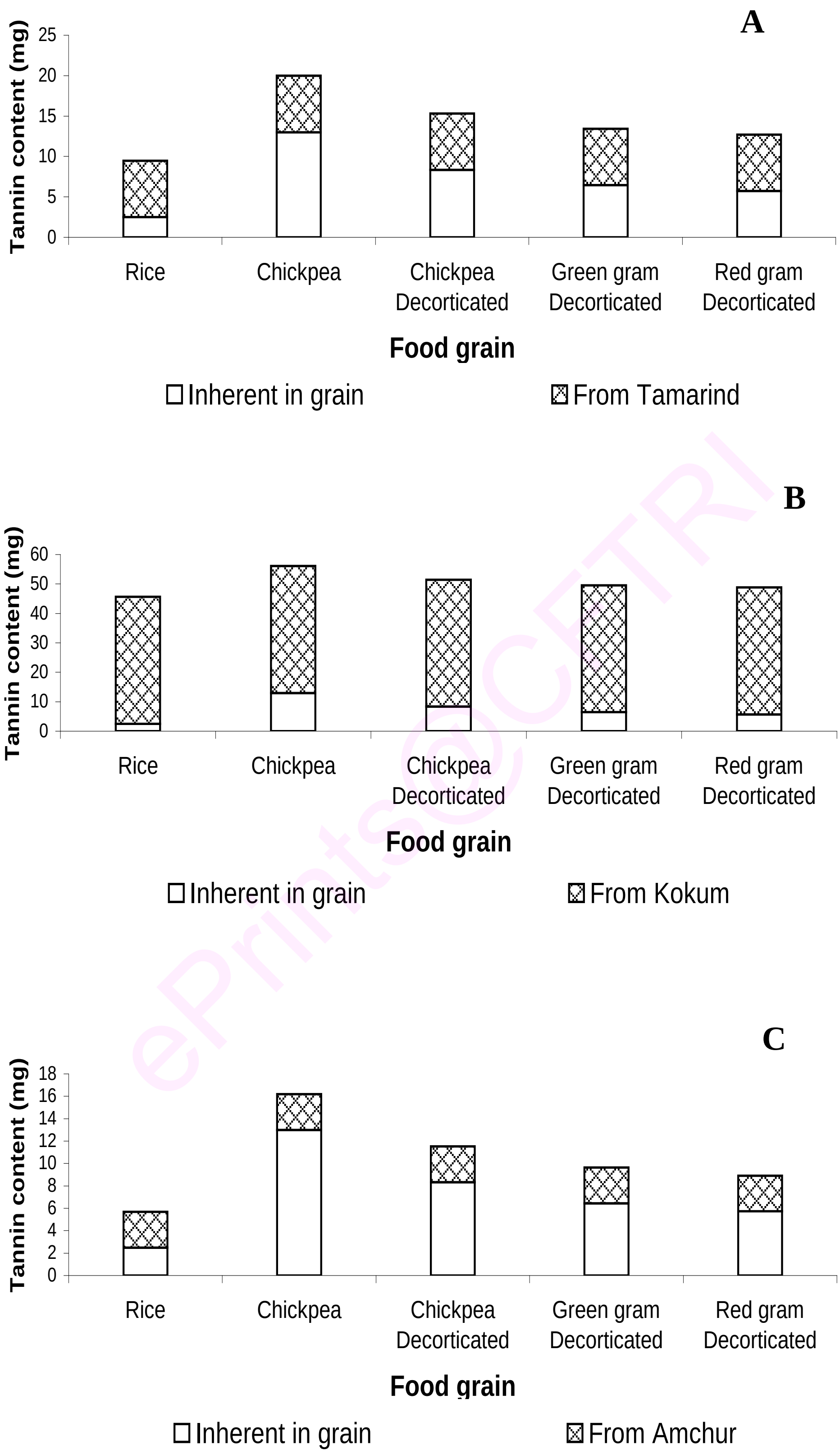


Fig. 9 Tannin concentration in food samples contributed by acidulants – tamarind (A), kokum (B) and amchur (C)

Among the food grains examined, whole chickpea seems to have derived the maximum beneficial effect from all the four food acidulants, especially when cooked, with reference to bioaccessibility of these two trace minerals. The particularly higher magnitude of positive influence of most of the acidulants on mineral bioaccessibility from rice observed in this study relative to other food grains could be attributed to the low titres of inherent factors such as phytate, tannin and calcium interfering with mineral absorption.

Zinc and iron bioaccessibility from whole meals:

Although zinc is mainly derived from staple cereals and pulses in a vegetarian diet, factors other than those inherently present in these grains may have an influence on the bioaccessibility of this mineral, since the grains are consumed as part of a complex meal, which consists of ingredients such as common salt, acidulants, vegetables, fat, and spices, in addition to these grains. As such, representative meals based on four different staple cereals as recommended by the Indian Council of Medical Research, India, (NIN, 1998) were examined for zinc bioaccessibility. Zinc and iron bioaccessibility values from these representative meals are presented in Table-5. The four meals had similar zinc concentrations, which ranged from 2.03 mg% in rice- based meal to 2.35 mg% in wheat- based meal. The iron content of the four meals ranged from 5.79 mg% in rice- based meal to 8.60 mg% in sorghum- based meal. Among the four meals examined, the rice- based meal had the highest bioaccessible zinc value, namely, 8.49%, while it was least in the sorghum- based meal (0.31%). In spite of similar zinc concentrations in the four meals, there was a wide variation in the bioaccessibility of zinc, which is a net result of the influence of multiple food ingredients associated with these meals. Thus, the rice- based meal had per cent bioaccessible zinc similar to that of the main ingredient cereal, rice [pressure cooked] (9.3%; Chapter II). However in the case of finger millet, the meal based on this cereal provided a higher percentage of bioaccessible zinc compared to pressure- cooked finger millet alone (3.1%; Chapter II), while wheat- and sorghum- based meals had much lower per cent bioaccessible zinc values than expected of the respective pressure- cooked cereals alone (8.5 and 9.5%, respectively; Chapter II).

Table- 5 Zinc and iron bioaccessibility from representative meals

Meal	Total zinc content of the meal(mg/100g)	Per cent zinc bioaccessibility	Total iron content of the meal(mg/100g)	Per cent iron bioaccessibility
Rice-based	2.03	8.49 ± 0.62	5.79	2.48 ± 0.21
Finger millet-based	2.10	5.77 ± 0.29	8.00	1.50 ± 0.05
Wheat-based	2.35	1.57 ± 0.22	6.54	4.70 ± 0.32
Sorghum-based	2.21	0.31 ±0.04	8.60	3.51 ± 0.17

Values are mean ± SEM of 5 replicates determined from freeze-dried meals

Iron bioaccessibility values from these meals ranged from 1.50% in finger millet- based meal to 4.70% in wheat- based meal. Similar to individual staple cereals (Chapter II), zinc bioaccessibility from the whole meals based on them was higher than that of iron, in the case of rice- and finger millet- based meals.

These results suggest that population whose staple cereal is either rice or finger millet probably derives more zinc from the daily diet than their counterparts who are dependent on either sorghum or wheat as the staple cereal, while the reverse trend is seen in the case of iron bioaccessibility, with wheat- or sorghum- based meals providing more than twice the bioaccessible iron contributed by either rice- or finger millet- based meals.

SUMMARY

Four common acidulants in Indian dietary, namely, citric acid, tamarind (*Tamarindus indica*), amchur (dry mango powder, *Mangifera indica*), and kokum (*Garcinia indica*) were examined for a possible influence on the bioaccessibility of zinc from selected food grains. Among the four acidulants examined, amchur and citric acid generally enhanced the bioaccessibility of zinc and iron from all the food grains studied. The increase in zinc bioaccessibility produced by citric acid was around 40% in rice and chickpea, while amchur produced around 60% increase in the same from decorticated green gram. This positive influence of acidulants on zinc bioaccessibility from food grains was seen both in the raw and cooked form. Among the food grains examined, whole chickpea seems to have derived the maximum beneficial effect from all the four food acidulants, especially when cooked, with reference to bioaccessibility of these two trace minerals. Tamarind and kokum, the other two acidulants tested, generally did not have a favourable influence on zinc and iron bioaccessibility. This lack of positive influence of these two acidulants on mineral availability could be attributable to the presence of significant amounts of tannin in them.

Zinc concentrations in the four whole meals tested ranged from 2.03 mg% in rice- based meal to 2.35 mg% in wheat- based meal. The iron content of the four meals ranged from 5.79 mg% in rice- based meal to 8.60 mg% in sorghum- based

meal. Among the four meals examined, the rice- based meal had the highest bioaccessible zinc value, while it was least in the sorghum- based meal. In spite of similar zinc concentrations in the four whole meals, there was a wide variation in the bioaccessibility of zinc, which is a net result of the influence of multiple food ingredients associated with these meals. The wheat- based meal had the highest per cent bioaccessible iron content, while the finger millet- based meal had the least percentage of bioaccessible iron.

GENERAL SUMMARY

General Summary

Cereals and legumes are staple foods in the diet of populations in the developing countries. These are also the main sources of minerals such as iron and zinc. However, mineral availability from these food grains is generally poor, due to the presence of phytic acid, tannin and dietary fibre components. While the effects of these constituents on iron absorption are well documented, their effects on zinc bioavailability are less known. In this context, a number of staple cereals and pulses commonly consumed in India were examined for zinc bioaccessibility. The bioaccessibility values of zinc and iron have also been examined in heat- processed food grains. In view of a possible interaction among minerals influencing their absorbability, the effect of presence of exogenous iron and calcium on zinc bioaccessibility from selected food grains has also been examined.

The much- practiced germination and fermentation of grains as encountered in Indian dietary have been examined for a possible beneficial influence on zinc and iron bioaccessibility. The influence of food acidulants on zinc bioaccessibility by virtue of their organic acid content has also been assessed in this investigation.

The highlights of the results of this investigation are listed below:

1. Several food grains commonly consumed in India were screened for zinc content and its bioaccessibility from the same was determined by equilibrium dialysis employing an *in vitro* simulated digestion procedure. The food grains examined included the cereals - rice (*Oryza sativa*), finger millet (*Eleusine coracana*), wheat (*Triticum aestivum*), maize (*Zea mays*), sorghum (*Sorghum vulgare*), and the pulses - chickpea (*Cicer arietinum*), green gram (*Phaseolus aureus*), red gram (*Cajanus cajan*), black gram (*Phaseolus mungo*), cowpea (*Vigna catjang*), and French bean (*Phaseolus vulgaris*).

2. The total zinc content of cereals ranged from 1.08 mg / 100 g in rice to 2.24 mg/ 100 g in sorghum. The zinc content of pulses was between 2.03 mg/ 100 g (whole chickpea) and 2.68 mg/ 100 g (decorticated chickpea). The bioaccessibility of zinc from pulses (27 - 56%) was generally higher than that from cereals (5.5 - 21.4%).

3. The iron content of cereals ranged from 1.32 mg% in rice to 6.51 mg% in sorghum, while that of pulses ranged from 3.85 mg% in decorticated green gram to 6.46 mg% in black gram. Bioaccessibility of iron was almost similar from both cereals and pulses examined and ranged from 4.13 to 8.05% in cereals and from 1.77 to 10.2 % in pulses.

4. Phytic acid content of these food grains ranged from 160 to 630 mg %. A significant negative correlation between inherent phytate and zinc bioaccessibility value was inferred in the case of pulses. However, the same was not evident when the bioaccessibility value was viewed in relation to phytate: zinc molar ratios.

5. In the case of iron, phytic acid content of the cereal grains produced a proportionate and statistically significant negative influence on its bioaccessibility.

6. The calcium content of the food grains tested ranged from 7.2 mg % in maize, to 325 mg % in finger millet. This inherent calcium had a negative influence on zinc bioaccessibility in cereals. Zinc bioaccessibility from the food grains was also negatively influenced by [phytate] x [calcium] / [zinc] molar ratio, which however was not statistically significant. Such an inverse relationship of calcium or of [phytate] x [calcium] / [iron] molar ratio was not evident on iron bioaccessibility.

7. Among the food grains tested, finger millet had the highest amount of tannin (2.12 g %) and similar high amounts were present in French beans (3.08 g %) and cowpea (2.21 g %). Tannin did not have any significant influence on zinc and iron bioaccessibility from cereals and pulses.

8. Legumes (especially whole) had higher dietary fibre content. While both insoluble and soluble fractions of the dietary fibre in the food grains generally interfered with zinc bioaccessibility, the insoluble fraction alone had this effect on iron bioaccessibility. Soluble fraction, on the other hand, had the opposite effect on iron bioaccessibility, more so in the case of cereals.

9. The lower collective negative influence of the various inherent factors on zinc bioaccessibility from pulses is consistent with the higher values of the same in these grains, relative to cereals. The extent of collective influence of the various inherent factors on iron bioaccessibility from cereals and pulses was essentially similar.

10. The negative correlation of inherent phytic acid with zinc and iron bioaccessibility was supported by enhanced bioaccessibility of these minerals observed upon partial removal of phytate from the food grains by treatment with fungal phytase.

11. Exogenous phytic acid showed a marginal decreasing effect on zinc bioaccessibility from the food grains studied, which was however not proportionate to the level added. Iron bioaccessibility values, however, were profoundly enhanced from all the food grains tested.

12. Influence of heat processing on the *in vitro* availability of zinc from these cereals and pulses was also examined. In general, zinc bioaccessibility from these food grains was considerably reduced upon domestic cooking (pressure-cooking or microwave heating). Zinc bioaccessibility was reduced to nearly half in rice, wheat, chickpea and green gram.

13. Iron bioaccessibility on the other hand, was significantly enhanced generally from all the food grains studied upon heat treatment. Thus, heat treatment of grains produced contrasting effect on zinc and iron bioaccessibility.

14. The influence of exogenously added iron and calcium at a level up to four times the inherent concentration on the bioaccessibility of zinc from selected food grains was examined. Presence of exogenous iron at levels tested here (up to five times the intrinsic level) did not severely inhibit zinc bioaccessibility from rice and decorticated green gram. The results also suggested that the negative effect of exogenous iron is probably discernible only when zinc: iron molar ratio exceeds 1:8, as encountered during supplemental iron regimen.

15. Exogenously added calcium did not influence the bioaccessibility of either zinc or iron from rice and wheat, even when added at levels five times those present inherently. This can be attributed to the fact that calcium *per se,* in the absence of additional phytate, may not have a negative effect on mineral availability.

16. The influence of exogenous soy protein, on zinc and iron bioaccessibility from representative cereals- rice and sorghum was also examined. Influence of common salt along with exogenous protein on the bioaccessibility of zinc and iron from these food grains has also been specifically examined. Exogenous protein (soy protein isolate) produced contrasting effects on zinc and iron bioaccessibility from the food grains studied here. While soy protein had a negative effect on iron bioaccessibility from these food grains, the same produced an enhancing effect on zinc bioaccessibility.

17. Exogenously added sodium chloride potentiated the positive effect of soy protein on zinc bioaccessibility, and effectively countered its negative effect on iron bioaccessibility.

18. Germination of green gram, chickpea and finger millet for 24 and 48 h significantly enhanced the bioaccessibility of iron; bioaccessibility of zinc was not beneficially affected. In the absence of any decrease in phytate content of the grains, reduction in tannin content during germination of the test grains could have contributed to the increase in the bioaccessibility of iron.

19. Fermentation of the batter of cereal-pulse combination as in the preparation of '*idli*' and '*dosa*' significantly enhanced the bioaccessibility of both zinc and iron, the extent of increase in the case of iron being still better. However, such beneficial influence of fermentation on zinc and iron bioaccessibility was not observed in the case of the cereal-pulse combination used for the preparation of '*dhokla*'.

20. Significant reduction of both phytate and tannin during fermentation of cereal-legume combinations of the '*idli'* and '*dosa'* batter must have contributed to the observed increase in mineral bioaccessibility.

21. Absence of any positive influence of fermentation on mineral bioaccessibility in the case of 'd*hokla'* batter could be attributed to the continued presence of significant amounts of phytate or any other protein that binds these micronutrients contributed by additional legumes - chickpea and green gram present in 'd*hokla'*.

22. Soaking, germination and fermentation of cereals and legumes (in which phytate is in the cotyledons) offers a practical household method to reduce inhibitors of mineral absorption especially phytic acid and tannin, thereby contributing to enhanced zinc and iron absorption. In fact, such processing of cereals and legumes as part of the daily culinary are in wide practice across the Indian subcontinent.

23. Four common acidulants in Indian dietary, namely, citric acid, tamarind (*Tamarindus indica*), amchur (dry mango powder, *Mangifera indica*), and kokum (*Garcinia indica*) were examined for a possible influence on the bioaccessibility of zinc from selected food grains. Amchur and citric acid generally enhanced the bioaccessibility of zinc and iron from all the food grains studied. The increase in zinc bioaccessibility produced by citric acid was around 40% in rice and chickpea, while amchur produced around 60% increase in the same from decorticated green gram. This positive influence of acidulants on zinc bioaccessibility from food grains was seen both in the raw and cooked form.

24. Among the food grains examined, whole chickpea seems to have derived the maximum beneficial effect from all the four food acidulants, especially when cooked, with reference to bioaccessibility of these two trace minerals.

25. Tamarind and kokum, the other two acidulants tested, generally did not have a favourable influence on zinc and iron bioaccessibility. This lack of positive influence of these two acidulants on mineral availability could be attributable to the presence of significant amounts of tannin in them.

26. Zinc concentrations in the four whole meals tested ranged from 2.03 mg% in rice-based meal to 2.35 mg% in wheat- based meal. The iron content of the four meals ranged from 5.79 mg% in rice- based meal to 8.60 mg% in sorghum- based meal.

27. Among the four meals examined, the rice- based meal had the highest bioaccessible zinc value, while it was least in the sorghum- based meal. In spite of similar zinc concentrations in the four whole meals, there was a wide variation in the bioaccessibility of zinc, which is a net result of the influence of multiple food ingredients associated with these meals.

28. The wheat- based meal had the highest per cent bioaccessible iron content, while the finger millet- based meal had the least percentage of bioaccessible iron.

Thus,

- ❖ Pulses are better providers of bioaccessible zinc compared to cereals
- ❖ Bioaccessibility of zinc from cereals and pulses commonly consumed in India is higher than that of iron
- ❖ Domestic cooking which involved pressure cooking or microwave cooking generally improved the bioaccessibility of iron, while that of zinc was significantly compromised; nevertheless, zinc bioaccessibility values from pressure- cooked pulses are superior to that of iron
- ❖ Germination of grains as commonly practiced in Indian households improves iron bioaccessibility
- ❖ Fermented food products – '*idli*' and '*dosa*' are good sources of bioaccessible zinc and iron
- ❖ Food acidulants – lime and amchur generally enhance both zinc and iron bioaccessibility

Bibliography

CHAPTER-I

Agte V, Joshi S, Khot S, Paknikar K and Chiplonkar S (1998): Effect of processing on phytate degradation and mineral solubility in pulses. *J.Food Sci.Technol*. 35: 330-332

Akhtar D, Begum N and Sattar A (1987): Effect of dietary phytate on bioavailability of iron. *Nutr.Res*. 7: 833-842

Ammerman CB, Baker DH and Lewis AJ (1995): Bioavailability of nutrients for animals. Academic Press, New York, 441

Anderson JJB (2000): Minerals. In: Krause's Food, Nutrition & Diet Therapy. Mahan LK and Escott-Stump S (Eds.): WB Saunders Co., Philadelphia, pp. 111 - 152

Azenha M and Vascondecelos M (2000): Assessment of the Pb and Cu *in vitro* availability in wines by jeans of speciation procedures. *Food Chem.Toxicol*. 38: 899 –912

Ballot D, Baynes RD, Bothwell TH, Gillooly M, MacFarlane BJ, MacPhail AP, Lyons G, Derman DP, Bezwoda WR, (1987): The effect of fruit juices and fruits on the absorption of iron from a rice meal. *Br.J.Nutr*. 57: 331-343

Beard JL, Weaver CM, Lynch S, Johnson CD, Dassenko S and Cook JD (1988): The effect of soybean phosphate and phytate content on iron bioavailability. *Nutr.Res*. 8: 345-352

Beard JL, Connor JR and Jones BC (1993): Iron in the brain. *Nutr.Rev*. 51:157-170

Behall KM, Scholfield DJ, Lee K, Powell AS, and Moser PB (1987): Mineral balance in adult men: Effect of four refined fibre. *Am.J.Clin.Nutr*. 46: 307

Bender AE (1989): Nutritional significance of bioavailability In: Southgate DAT, Johnson IT, Fenwick GR (Eds): Nutrient availability: Chemical and biological aspects. Special publication No 72, Royal Society of Chemistry, Cambridge, pp 3-9

Berdaneir CD (1997): Trace minerals. In: Advanced Nutrition-Micronutrients, CRC press Washington DC, pp. 187-192.

Berg JM and Shi Y (1996): The galvanization of biology: A growing appreciation for the roles of zinc. *Science* 271: 1081-1085

Black MM (1998): Zinc deficiency and child development. *Am.J.Clin.Nutr*. 68 (Suppl): 464S-469S

Bothwell TH, Charlton RW, Cook JD and Finch CA (1979): Iron metabolism in man. Blackwell Publications, London, pp. 431-433

Bothwell TH, Baynes RD, MacFarlane BJ and MacPhail AP (1989): Nutritional iron requirements and food iron absorption. *J.Int.Med*. 226: 357-365

Breskin MW, Worthington-Roberts BS, Knopp RH, Brown ZP, Lovie B Mottet NK and Mills JL (1983): First trimester serum zinc concentration in human pregnancy. *Am.J. Clin.Nutr*. 38: 943-953

Brown KH, Wuehler SE, and Peerson JM (2001): The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food Nutr.Bull*. 22: 113-125

Brown KH, Peerson JM, Rivera J and Allen LH (2002): Effect of supplemental zinc on growth and serum zinc concentrations of pre-pubertal children: A meta analysis of randomized controlled trials. *Am.J.Clin.Nutr*. 75: 1062-1071

Brune M, Rossander-Hulten L, and Hallberg L (1984): Iron absorption: No intestinal adaptation to a high phytate diet. *Am.J.Clin.Nutr*.49: 542

Brune M, Rossander-Hulten L, Gleerup A, and Sandberg AS (1992): Iron absorption from bread in humans: Inhibiting effects of cereal fibre, phytate and inositol phosphates with different numbers of phosphate groups. *J.Nutr.* 122: 442-449

Calesnick B and Dinan AM (1988): Zinc deficiency and zinc toxicity. *Am.Fam.Physician*. 37: 267-270

Campbell-Brown M, Ward RJ, Haines AP, North WRS, Abraham R and McFayden IR (1985): Zinc and copper in Asian pregnancies - Is there evidence for a nutritional deficiency? *Br.J.Obstet.Gyneco*.l92: 975-985

Carpenter CE and Mahoney AW (1992): Contributions of heme and nonheme iron to Human Nutrition. *Crit.Rev.Food.Sci.Nutr*. 31: 333-367

Champagne ET, Rao RM, Liuzzo JA Robinson JW, Gale RJ and Miller F (1985): The interactions of minerals proteins and phytic acid in Rice Bran. *Cereal Chem*.62: 231

Chesters JK (1989): Biochemistry of zinc in cell division and tissue growth. In: Mills CF (Ed): Zinc in human biology. Springer, London, pp.109–118

Coleman JE (1992): Zinc proteins: Enzymes storage proteins transcription factors and replication proteins. *Ann.Rev.Biochem*. 61: 897-946

Conrad ME and Crosby WH (1962): The natural history of iron deficiency induced by phlebotomy. *Blood* 20: 173-185

Cook JD, Layrissse M, Martinez-Torres C, Walker R, Monsen E and Finch CA (1972): Food iron absorption measured by an extrinsic tag. *J.Clin.Invest*. 51: 805-815

Cook JD and Monsen ER (1976): Food iron absorption in human subjects: III. Comparison of the effect of animal proteins on non-heme iron absorption. *Am.J.Clin. Nutr*. 29: 859-867

Cosgrove DJ (1980): Studies in inorganic chemistry of inositol phosphates: their chemistry, biochemistry and physiology. New York: Elsevier Scientific publishing company.

Cossack ZT and Prasad AS (1983): Effect of protein source on the bioavailability of zinc in human subjects. *Nutr.Res.* 3: 23-31

Cousins RJ (1979): Regulatory aspects of zinc metabolism in liver and intestine .*Nutr.Rev*. 37: 97-103

Craig WJ, Balbach L and Vyhmeister N (1984): Zinc Bioavailability and Infant Formulas *Am.J.Clin.Nutr*. 39: 981-983

Davies NT and Nightingale R (1975): The effect of phytate on intestinal absorption and secretion of zinc copper iron and magnesium in rats *Br. J .Nutr*. 34: 243-58

Davies NT and Olpin SE (1979): Studies on the phytate : zinc molar contents in diets as A determinant of zinc availability to young rats. *Br.J.Nutr*. 41: 591-603

Deshpande SS, Sathe SK and Salunkhe DK (1984): Chemistry and safety of plant polyphenols. In: Friedman M (Ed): Nutritional and toxicological aspects of food safety. Plenum Press, New York, pp. 457-495

Disler PB, Lynch SR, Charlton RW, Torrance JD and Bothwell TH (1975): The effect of tea on iron absorption. *Gut* 16: 193-200

Drews LM, Keis C and Fox HM (1979): Effect of dietary fibre on copper zinc and magnesium utilization by adolescent boys. *Am.J.Clin.Nutr*. 32: 1893-1898

Duhan A, Khetarpaul N and Bishnoi S (2002): Content of phytic acid and HCl-extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. *Food.Chem*.78: 9 -14

Duhan A, Khetarpaul N and Bishnoi S (2004): HCl-extractability of zinc and copper as affected by soaking, dehulling, and cooking and germination of high yielding pigeon pea cultivars. *J.Food Comp.Anal.* 17: 597–604

Ellis R, Morris ER, Hill AD and Smith JC Jr. (1981): Phytate: zinc molar ratio of regular ovo-lacto-vegetarian and soya meat substitute hospital diets. *Fed.Proc*. 40: 938

Emes JH and Arthur D (1975): The site of zinc absorption. In: The rat small intestine *Proc.Soc.Exp.Biol.Med.*148: 86-88

Evans GW, Grace CI and Votava HJ (1975): A proposed mechanism of zinc absorption in the rat. *Am.J.Physiol*. 228: 501-505

Fairweather-Tait SJ (1987): The concept of bioavailability as it relates to iron nutrition *Nutr.Res*. 7: 319-325

Fairweather-Tait SJ (1988): Zinc in human nutrition. *Nutr.Res.Rev.*1: 23-37

Fairweather-Tait SJ (1992): Bioavailability of trace elements. *Food Chem.* 43: 213-217

Fairweather-Tait S and Wright AJA (1990): The effects of sugar beet fiber and wheat bran on iron and zinc absorption in rats *Br. J. Nutr.* 64:547-552

Falchuk K (1998): The molecular basis for the role of zinc in developmental biology *Mol.Cell.Biochem.* 188: 41-48

Farah DA, Hall MJ, Mills PR and Russell RI (1984): Effect of wheat bran on zinc absorption. *Hum.Nutr.Clin.Nutr*. 38: 433-441

Florentino RF (2003): The burden of iron deficiency and anemia in Asia: Challenges in prevention and control. In: Proceedings of IX Asian Congress of Nutrition, pp. 313-318

Forbes RM and Erdman Jr. JW (1983): Bioavailability of trace mineral elements. *Ann. Rev.Nutr*. 3: 213-231

Forbes RM and Yohe M (1960): Zinc requirement and balance studies with the rat. *J.Nutr*. 70: 53-57

Forbes RM, Erdman JW, Parker HM, Kondo HM and Ketelsen SM (1983): Bioavailability of zinc in coagulated soy protein (Tofu): to rats and effects of dietary calcium at a constant phytate/zinc ratio *J. Nutr*. 113:205-210

Fox MRS and Tao SH (1989): Anti-nutritive effects of phytate and other phosphorylated derivatives. In Nutritional Toxicology, Hathcock JN (Ed): Academic press,New York 59- 96

Fritz JC, Pla GW, Harrison BW and Clarke GA (1975): Estimation of the bioavailability of Iron. *Anal.Chem.* 58: 902-905

Frolich W and Asp NG (1980): Mineral bioavailability and cereal fibre. *Am.J.Clin.Nutr*. 33: 2397

Frolich W and Nyman M (1988): Minerals, phytate and dietary fiber in different fractions of oat-grain. *J.Cereal Sci*. 7: 73- 82

Gangloff MB, Raymond MS, Glahn P, Miller DD and Van Campen DR (1996): Assessment of iron availability using combined *in vitro* digestion and Caco-2 cell Culture. *Nutr.Res.* 16: 479-487

Garcia MN, Flowers C and Cook JD (1996): The Caco-2 cell culture system can be used as a model to study food iron availability. *J.Nutr*. 126: 251–258

Geders JM, Brown TD and Crews MG (1981): Effect of magnesium on zinc absorption. *Fed.Proc*. 40: 938 (Abstr)

Ghishan FK, Said HM, Wilson PC, Murrell JE and Greene HL (1986): Intestinal transport of zinc and folic acid: A mutual inhibitory effect. *Am.J.Clin.Nutr.* 43: 258-262

Gibson RS (1994): Content and bioavailability of trace elements in vegetarian diets. *Am.J.Clin.Nutr.* 59 (Suppl.5):1223S-12232S

Glahn RP, Wien EM, Van-Campen DR and Miller DD (1996): Caco-2 cell iron uptake from meat and casein digests parallels i*n vivo* studies: Use of a novel i*n vitro* method for rapid estimation of iron bioavailability. *J.Nutr.* 126: 332–339

Gleerup A, Rossander-Hulten L, Gramatkovski E and Hallberg L (1995): Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am.J.Clin.Nutr.* 61: 97-104

Greger JL, Zaikis SC, Abernathy RP, Bennet OA and Huffman J (1978): Zinc, nitrogen, copper, iron and manganese balance in adolescent females fed two levels of zinc. *J. Nutr*. 108: 1449-1456

Greger JL and Mulvaney J (1985): Absorption and tissue distribution of zinc iron and copper by rats fed diets containing lactalbumin soy and supplemental sulfur-containing amino acids. *J.Nutr.* 115: 200-210

Guthrie BE and Robinson MF (1978): Zinc balance studies during wheat bran supplementation. *Fed.Proc.* 37: 254 (Abstr.)

Hallberg L (1981): Bioavailability of dietary iron in man. *Ann.Rev.Nutr*. 1: 123-147

Halberg L and Solvell L (1967): Absorption of hemoglobin iron in man. *Acta Med. Scand.* 181: 335-354

Hallberg L, Bjorn-Rasmussen E, Garby L, Pleehachinda R and Suwanik R (1978): Iron absorption from South East Asian diets and the effect of iron fortification. *Am. J. Clin. Nutr.* 31: 1403-1408

Hallberg L, Rossander-Hulten L and Skanberg AB (1987): Phytates and the inhibitory effect of bran on iron absorption in man. *Am.J.Clin.Nutr.* 45: 988-996

Hallberg L, Brune M and Rossander-Hulten L (1989): Iron absorption in man: Ascorbic acid and dose-dependent inhibition by phytate. *Am.J.Clin.Nutr*. 49: 140- 144

Hallberg L, Rossander-Hulten L, Brune M and Gleerup A (1992): Calcium and iron absorption: Mechanism of action and nutritional importance. *Eur.J.Clin.Nutr*. 46: 317-327

Hallberg L, Rossander-Hulten L, Brune M and Gleerup A (1993): Inhibition of heme-iron absorption in man by calcium *Br. J. Nutr*. 69: 533-540

Hallberg L, Rossander-Hulten L and Gramatkovski E (1997): Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am.J.Clin. Nutr*. 66: 347-356

Hambidge KM (2000): Human zinc deficiency. *J.Nutr*.130:1344S-1349S

Hambidge KM, Krebs NF, Jacobs MA, Favier A, Guyette L and Ilke DM (1983): Zinc nutritional status during pregnancy: A longitudinal study. *Am.J.Clin.Nutr*.37: 429-442

Harland BF and Morris ER (1995): Phytate: A good or a bad food component? *Nutr. Res*. 15: 733-754

Hazell T and Johnson IT (1987): *In vitro* estimation of iron availability from a range of plant foods: Influence of phytate, ascorbate and citrate. *Br. J. Nutr*. 57: 223-234

Heinrich HC (1987): Intestinal absorption of 5g Fe from neutron-activated commercial oral iron(III): citrate and iron(III): hydroxidepolymaltose complexes in man. *Drug Res.* 37: 105-107

Hill CH and Matrone G (1970): Chemical parameters in the study of *in vivo* and *in vitro* interactions of transition elements. *Fed.Proc.* 29:1474-1481

Hong C, Wu J, Zhu S and Liu Z (1994): The effect of vitamin A on iron metabolism of mouse and its mechanisms. *Acta Nutrimenta Sinica* 16: 138-143

House WA (1999): Trace element bioavailability as exemplified by iron and zinc. *Field Crops Research* 60: 115-141

House WA, Van Campen DR and Welch RM (1997): Dietary methionine status and its relation to the bioavailability to rats of zinc in corn kernels with varying methionine content. *Nutr.Res.* 17: 65-76

Hunt JR, Johnson PE and Swan PB (1987): Dietary conditions influencing relative zinc availability from foods to the rat and correlations with *in vitro* measurements. *J.Nutr.* 117: 1913-1923

Ine PM, Wauben and Atkinson SA (1999): Calcium does not inhibit iron absorption or alter iron status in infant piglets adapted to a high calcium diet. *J.Nutr.* 129: 707–711

Inglet GE (1983): Nutritional bioavailability of zinc. *ACS Symposium Series* 210, Am.Chem.Soc.Press, Washington DC

James B, McClunga P, Stahlb CH, Marchitellia LJ, Orales-Martineza N, Mackina KM, Younga AJ and Scrimgeoura AG (2005): Effects of dietary phytase on body weight gain body composition and bone strength in growing rats fed a low-zinc diet. *J.Nutr. Biochem*. (*in press*)

Johnson MA, Baier M and Greger JL (1981): Effect of dietary tin on human mineral metabolism. *Fed.Proc*. 40: 950 (Abstr.)

Kaur M and BL Kawatra (1999): Molar ratios to predict zinc availability from domestically processed rice bean (*Vigna umbellata*):. *J.Food Sci.Technol.* 36: 548-550

Keating JN, Wada L, Stokstad EL and King JC (1987): Folic acid: effect on zinc absorption in humans and in the rat. *Am.J.Clin.Nutr*.46 (5): 835-839

Kelsay JL (1983): In: Nutritional bioavailibility of zinc. Inglett GE (Ed.): American Chemical Society, Washington DC

Kim MZ and Atallah MT (1993): Intestinal solubility and absorption of ferrous iron in growing rats are affected by different dietary pectins. *J.Nutr.* 123: 117-124

Kim M, Atallah MT, Amarasiriwardena C and Barnes R (1996): Pectin with low molecular weight and high degree of esterification increases absorption of ^{58}Fe in growing rats. *J.Nutr*. 126: 1883-1890

King JC and Keen CL (1999): In: Modern nutrition in health and disease. 9th Edn: Shills ME, Olson JA and Shike M and Ross AC (Eds.): Williams and Wilkins,Baltimore,pp 223-239

King JC and Turnland JR (1989): Human zinc requirements. In: Zinc in Human Biology. Mills CF (Ed): Springer-Verlag, London, pp. 337-379

Larsson M, Rossander-Hulten L, Sandstrom B and Sandberg AS (1996): Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. *Br.J.Nutr*. 76: 677-688

Lawman JT and Krivit W (1963): New *in vivo* tracer method with the use of non-radioactive isotopes and activation analysis. *J.Lab.Clin.Med*. 61: 1042

Layrisse M, Martinez-Torrse C and Roche M (1968): The Effect of interaction of various foods on iron absorption. *Am.J.Clin.Nutr*. 21: 1175-1183

Layrisse M and Martinez-Torrse C (1972): Model for measuring dietary absorption of heme iron-test with a complete meal. *Am.J.Clin.Nutr*. 25: 401-411

Layrisse M, Garco Aa-Casal MN, Solano L, Baron MA, Arguello F, Llovera D, Ramo-Arez J, Leets I. and Tropper E (1997): The role of vitamin A on the inhibitors of non-heme iron absorption: Preliminary results. *J.Nutr.Biochem.* 8: 61-67

Lei X, Ku PK, Miller ER, Ullrey DE and Yokoyama MT (1993): Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. *J.Nutr*. 123: 1117-1123

Lestienne I, Icard-Verniere C, Mouquet C, Picq C and Treche S (2005): Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* 89: 421-425

Lind T, Lonnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekstrom EC and Persson LA (2003): A community based randomized controlled trial of iron and zinc supplementation in Indonesian infants: Interactions between iron and zinc. *Am.J. Clin.Nutr*. 77: 883-890

Lo GS, Settle SL, Steinke FH and Hopkins DT (1981): Effect of phytate/zinc molar ratio on zinc bioavailibility. *Fed.Proc*. 40: 938 (Abstr):

Lonnerdal B, Sandberg AS, Sandstrom B and Kunz C (1989): Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J.Nutr.*119: 211

Lopez HW, Duclos V, Coudray C, Krespine V, Feillet-Coudray C, Messager A, Demigne C and Christian R (2003): Making bread with sour dough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutrition* 19: 524–530

Luten J, Crews H, Flynn A, Dael PV, Kastenmayer P, Hurrel R, Deelstra H, Shen LH, Fairweather- Tait S, Hickson K, Farre R, Schlemmer U and Frohlich W (1996): Inter-laboratory trial on the determination of the *in vitro* iron dialyzability from food. *J Sci Food Agric.* 72: 415-424

Marfo EK, Simpson BK, Idowu JS and Oke OL (1990): Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea and Soybean. *J.Agric.Food Chem.* 38: 1580-1585

Matseshe JW, Phillips SF, Malagelada JR and McCall JT (1980): Recovery of dietary iron and zinc from the proximal intestine of healthy man: Studies of different meals and supplements. *Am.J.Clin.Nutr.* 33:1946-1953

Mejia LA, Hodges E and Rucker RB (1979): Role of vitamin A in the absorption retention and distribution of iron in the rat. *J.Nutr.* 109: 127-137

Mejia LA and Chew F (1988): Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *Am.J.Clin.Nutr.* 48: 595-600

Miller DD, Schricker BR, Rasmussen BS and Van Campen DR (1981): An *in vitro* method for estimation of iron availability from meals. *Am.J.Clin.Nutr.* 34: 2248-2256

Monsen ER and Cook JD (1976): Food iron absorption in human subjects: IV. The effects of calcium and phosphate salts on the absorption of non-heme iron. *Am.J.Clin.Nutr.* 29: 1142-1148

Moore CV and Dubach R (1951): Observations on the absorption of iron from foods tagged with radio iron. *Trans.Assoc.Am.Physicians.* 64: 245-256

Moore CV (1968): The absorption of iron from foods. In: Occurance, Causes and Prevention of Nutritional Anemias. Gblix (Ed.):, Uppsala, Almqvist and Wiksell, pp. 92-102

Morris ER and Ellis R (1976): Isolation of mono ferric phytate from wheat bran and its biological value as an iron source to the rat. *J.Nutr*. 106: 753-760

Morris ER and Ellis R (1980): Effect of dietary phytate/ zinc molar ratio on growth and bone zinc response of rats fed semi-purified diets. *J.Nutr*. 110: 1037-1045

Morris ER and Ellis R (1983): Dietary phytate / zinc molar ratio and zinc balance in humans. In: Inglett GE (Ed): Nutritional bioavailability of Zinc. *Am.Chem.Soc.*, Washington DC, pp.159-172

Navert D and Sandstrom B (1985): Reduction of the phytate content of bran by leavening in bread and its effects on zinc absorption in man. *Br.J.Nutr*. 53: 47-53

Nolan KB, Duffin PA and McWeeny DJ (1987): Effects of phytate on mineral bioavailability *in vitro* studies on Mg^{++}, Ca^{++}, Fe^{++}, Cu^{++} and Zn^{++} (also Cd^{++}): solubilities in the presence of phytate. *J.Sci.Food Agric.* 40: 79- 86

Oberleas D and Prasad AS (1975): Factors affecting zinc homeostasis. In: Prasad A S (Ed): Trace Elements in Human Health and Disease. Vol. I, Zinc and copper. Academic Press, New York, pp.155-162

Oberleas D and Harland BF (1981): Phytate content of foods: Effect on dietary zinc bioavailability. *J.Am.Dietet.Assoc.* 79: 433–436

Obizoba JC (1981): Zinc and copper metabolism of human adults fed combinations of corn, wheat, beans, rice and milk containing various levels of phytate. *Fed.Proc.* 40: 950

O’Brien OK, Zavaleta N, Caulfield LE, Wen J and Abrams SA (2000): Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. *J.Nutr*.130: 2251-2255

O’Dell BL (1979): In: Soy Protein and Human Nutrition. Wilcke H, Hopkins DT and Waggle DH (Eds):, Academic Press, New York, pp.87-207

O’Dell BL (1984): Bioavailibility of trace elements. *Nutr.Rev*. 429: 301-308

O'Dell BL and Savage JE (1972): Evaluation of zinc availability in foodstuffs of plant and animal origin. *J.Nutr*. 102: 653-660

Olivares M, Pizarro F, Pineda O, Name JJ, Hertrampf E and Walter T (1997): Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in humans. *J.Nutr*. 127: 1407-1411

Parul C, Khatry SK, LeClerq SC, Shrestha SR, Kimbrough-Pradhan E and West KP Jr. (2001): Iron and zinc interactions among pregnant Nepali women. *Nutr.Res*. 21: 141-148

Payton KB, Flanagan PR, Stinson EA, Chodirker DR, Chamberlain MJ and Valberg LS (1982): Technique for determination of human zinc absorption from measurement of radioactivity in a fecal sample or the body. *Gastroenterology* 83:1264-1270

Persson H, Turk M, Nyman M and Sandberg AS (1998): Binding of Cu^{++}, Zn^{++} and Cd^{++} to inositol tri-, tetra-, penta- and hexa- phosphates. *J.Agric.Food Chem.* 46: 3194-3200

Platt SR and Clydesdale FM (1987): Mineral binding characteristics of lignin, guar gum, cellulose, pectin and neutral detergent fiber under simulated duodenal pH conditions. *J.Food.Sci*. 52: 1414

Prabhavathi T and Rao BSN (1979): Effects of domestic preparation of cereals and legumes on ionisable iron. *J.Sci.Food Agric*. 30: 597-602

Prasad AS, Halstead JA and Nandini M (1961): Syndrome of iron deficiency anemia, hepatosplenolomegaly, dwarfism and geophagia. *Am.J.Med*. 31: 532-546

Prasad AS, Miale A, Faird Z, Sandstead HH, Schulert AR and Darby WJ (1963): Biochemical studies on dwarfism, hypogonadism and anemia. *Arch.Intern.Med*.111: 407-428

Prasad AS, Rabbani P, Abassi A, Bowersox E and Spivey–Fox MR (1978): Experimental zinc deficiency in humans. *Ann.Intern.Med.* 89: 483-490

Prasad AS (1981): Zinc deficiency in human subjects *Prog.Clin.Biol.Res*. 77:165-177

Rao BSN and Prabhavathi T (1978): An *in vitro* method for predicting the availability of iron from foods. *Am.J.Clin.Nutr*. 31: 169-195

Rao BSN, Vijayasarathi C and Prabhavathi T (1983): Iron absorption from habitual diets of Indians studied by extrinsic tag technique. *Indian J.Med.Res*. 77: 648-657

Reddy MB and Cook JD (1997): Effect of calcium intake on nonheme-iron absorption from a complete diet. *Am.J.Clin.Nutr*. 65: 1820-1825

Reddy MB, Hurrell RF, Juillerat MA and Cook JD (1996): The influence of different protein sources on phytate inhibition of non-heme iron absorption in humans. *Am. J. Clin.Nutr*. 63: 203-207

Reinhold JG, Nasr K, Lahimgarzadeh A and Hedayati H (1973): Effects of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus and nitrogen in man. *Lancet* 1: 283-288

Reinhold JG, Prasad AS, Karimian N, Hammick JW and Ismail-Beigi F (1974): Availibility of zinc in leavening wheat bread as measured by solubility and uptake by rat intestine *in vitro*. *J.Nutr* 104: 976-982

Reinhold JG, Ferrari B, Abadi P and Ismail-Beigi F (1976): Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption of wheat bread. *J.Nutr*.106: 493-503

Roig MJ, Alegri A, Barbera R, Farre R and Lagarda MJ (1999): Calcium bioavailability in human milk, cow's milk and infant formulas: Comparison between dialysis and solubility methods. *Food Chem.* 65: 353-357

Rossander L (1987): Effect of Dietary fiber on iron absorption in man. *Scand.J.Gastro-enterol*. 22: 68

Rossander L, Hallberg L, Bjorn-Rasmussen E (1979): Absorption of iron from breakfast meals. *Am.J.Clin.Nutr*. 32: 2484-2489

Salgueiro J, Zubillaga M, Lysionek A, Sarabia M, Caro R, De-Paoli J, Hager A, Weill R and Boccio J (2000): Zinc as an essential micronutrient: A review. *Nutr.Res.* 20:737-755

Salovaara S, Sandberg AS and Andlid T (2002): Organic acids influence iron uptake in the human epithelial cell line Caco-2. *J.Agric.Food Chem*. 50: 6233- 6238

Sandberg AS and Svanberg U (1991): Phytate hydrolysis by phytase in cereals: Effects on *in vitro* estimation of iron availability. *J.Food Sci. 56*: 1330 -1333

Sandstead H, Klevay L, Munoz J, Jacob R, Logan G Jr.,Dintzis F, Inglett G and Shuey W (1978): Human zinc requirements: Effects of dietary fibre on zinc metabolism. *Fed.Proc*. 37: 254 (Abstr)

Sandstrom B, Almgren A, Kivistoe B and Cederblad A (1987): Zinc absorption in humans from meals based on rye, barley, oatmeal triticale and whole wheat. *J.Nutr*. 117: 1898-1902

Sandstrom B (1989): Zinc and dietary fiber. *Scand.J.Gastroenterol*. 22: 190

Sayers MH, Lynch SR, Jacobs P, Charlton RW, Bothwell TH, Walker RB and Mayet F (1973): The effect of ascorbic acid supplementation on the absorption of iron on maize, wheat and soya. *Br.J.Haematol*. 24: 209-218

Schlemmer U, Muller H and Ki DJ (1995): The degradation of phytic acid in legumes prepared by different methods. *Eur.J.Clin.Nutr*. 49 (Suppl 3): S207-S210

Schultink W, Merzenich M Gross R, Shrimpton R and Dillon D (1997): Effects of iron- zinc supplementation on the iron, zinc and vitamin A status of anaemic pre-school children in Indonesia. *Food Nutr.Bull*. 81: 311-317

Scott ML and Ziegler TR (1963): Evidence of natural chelates which aid in the utilization of zinc by chicks. *J.Agric.Food Chem*. 11: 123-125

Scrimshaw NS (1994): The consequences of hidden hunger for individuals and societies. *Food Nutr.Bull*. 15: 3-24

Shahkallili Y, Finot PA, Hurrel R and Abia R (1990): Effects of food rich in polyphenols on nitrogen excretion in rats. *J.Nutr*.120: 346-352

Shankar AH and Prasad AS (1998): Zinc and immune function: The biological basis of altered resistance to infection. *Am.J.Clin.Nutr*. 68 (Suppl): 447S-463S

Siqueira EM, Arruda SF, de Sousa LM and de Souza EM (2001): Phytate from an alternative dietary supplement has no effect on the calcium, iron and zinc status in undernourished rats. *Arch.Latinoam.Nutr*. 51: 250-257

Smith KT and Cousins RJ (1980): Quantitative aspects of zinc absorption by isolated vascularly perfused rat intestine. *J.Nutr*. 110: 316-323

Snedeker SM and Greger JL (1983): Effect of dietary protein sulfur amino acids and phosphorus on human trace element metabolism. *J.Nutr*. 113: 644-652

Solomons NW (1982): Biological availability of zinc in humans *Am.J.Clin.Nutr*. 35:1048-1075

Solomons NW (1986): Competitive interaction of iron and zinc in the diet: Consequences for human nutrition. *J.Nutr*. 116: 927-935

Solomons NW, Pineda O, Viteri F and Sandstead HH (1983): Studies on bioavailability of zinc in humans: V. Mechanism of the intestinal interaction of non-heme iron and zinc. *J.Nutr*.113: 337-349

Southgate DAT (1989): Conceptual issues concerning the assessment of nutrient bioavailability. In: Nutrient availability: Chemical and Biological Aspects. Southgate DAT, Johnson IT and Fenwick GR (Eds): Special Publication No.72, Royal Society of Chemistry, Cambridge, pp.10-12

Strain JJ and Cashman KD (2003): Minerals and trace elements.In:Introduction to Human Nutrition.Gibney MJ, Vorster HH and Kok FJ(Eds):Blackwell Science Ltd UK,pp.195

Svanberg U, Lorri W and Sandberg AS (1993): Lactic fermentation of non-tannin and high-tannin cereals: Effects on *in vitro* estimation of iron availability and phytate hydrolysis. *J.Food Sci*. 58: 408-412

Swanson CA, Reamer DC, Veillon C and Levander OA (1983): Intrinsic labeling of chicken products with a stable isotope of selenium (^{76}Se): *J.Nutr*. 113: 793-799

Tapiero H and Tew KD (2003): Trace elements in human physiology and pathology: zinc and metallothioneins. *Biomed.Pharmacother*. 57: 399- 411

Todd WR, Elvehjem CA and Hart EB (1934): Zinc in the nutrition of the rat *Am.J.Physiol*. 107: 146-156

Torre M, Rodriguez AR and Saura–Calixto F (1991): Effect of dietary fibre and phytic acid on mineral availability. *Crit.Rev.Food Sci.Nutr*. 30: 1-22

Turnland J (1991): Bioavailability of dietary minerals to humans: The stable isotope approach. *Crit.Rev.Food Sci.Nutr*. 30: 387-396

Vallee BL and Falchuk KH (1993): The biochemical basis of zinc physiology. *Physiol. Rev. 73*: 79-118

Van Campen DR (1969): Copper interferences with the intestinal absorption of zinc-65 by rats. J Nutr. 97:104-108

Van de Vijver LPL, Kardinaal AFM, Charzewska J, Rotily M, Charles P, Maggiolini M, Ando S, Vaananen K, Wajszczyk B, Heikkinen J, Deloraine A and Schaafsma G (1999): Calcium intake is weakly but consistently negatively associated with iron status in girls and women in six European countries. *J.Nutr*. 129: 963–968

Van Soest PJ and Jones LHP (1988): Analysis and classification of dietary fiber. In: Trace Element in Analytical Chemistry in Medicine and Biology. Bratter P and Schramel T (Eds.): Walter De Gruyter, New York, and pp.351

Walravens PA and Hambidge KM (1976): Growth of infants fed a zinc supplemented formula. *Am.J.Clin.Nutr.* 29: 1114-1121

Welch RM and House WA (1984): Factors affecting the bioavailability of mineral nutrients in plant foods. In: Crops as Sources of Nutrients for Humans. Welch RM and Gabelman WH (Eds.): American Society of Agronomy, Madison, Special Publication No.48: 37-54

Welch RM, House WA and Van-Campen (1977): Effects of oxalic acid on availability of zinc from spinach leaves and zinc sulfate to rats. *J.Nutr*. 107: 929-933

West CE and Eilander A (2001): Bioefficacy of carotenoids. *Sight Life* 2: 6-10

White HS and Gynne TN (1971): Utilization of inorganic elements by young women eating iron fortified foods. *J.Am.Diet.Assoc.* 59: 27-33

WHO (1996): Zinc. In: Trace Elements in Human Nutrition and Health. World Health Organization, Geneva, pp. 72-104

WHO (2002): Quantifying selected major risks to health. In: The World Health Report -2002, WHO, Geneva

WHO (2002a): http://wwwwhoint/nut/idahtm Sept 25

Wienk KJ, Mark JJ and Beynen AC (1999): The concept of iron bioavailability and its assessment. *Eur.J.Nutr*. 38: 51-75

Yonekar L and Suzuki H (2003): Some polysaccharides improve zinc bioavailability in rats fed a phytic acid-containing diet. *Nutr.Res.* 23: 343-355

Zhang D, Hendricks DG, Mahoney AW, Yu Y, Thannoun AM and Sisson DV (1991): Bioavailability of total dietary iron from beef and soy protein isolate alone or combined in anemic and healthy rats. *Cereal Chem.* 68: 194-200

CHAPTER-II

Anderson JJB (2000): Minerals. In: Krause’s Food, Nutrition & Diet Therapy. Mahan LK and Escott-Stump S (Eds.):, WB Saunders Co., Philadelphia, pp. 111 - 152

Asp NG, Johansson CG, Hallmer H, and Siljestrom M (1983): Rapid enzymatic assay of insoluble and soluble dietary fiber. *J.Agric.Food Chem*. 31: 476-482

Bindra GS, Gibson RS and Thompson LU (1986): Phytate x calcium / zinc ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. *Nutr.Res*. 6: 475 – 483

Brune M, Rossander L and Hallberg L (1989): Iron absorption: no intestinal adaptation to a high phytate diet. *Am.J.Clin.Nutr*. 49: 542 – 545

Brune M, Rossander – Hulthen L, Gleerup A, and Sandberg A-S (1992): Iron absorption from bread in humans. Inhibiting effects of cereal fibre, phytate and inositol phosphates with different numbers of phosphate groups. *J. Nutr* 122: 442 -449

Carbonaro M, Lombardi-Boccia G and Carnovale E (1995): Influence of the method of protein extraction on the *in vitro* evaluation of mineral dialysability from legumes. *Food Chem.* 53, 249-252

Chitra U, Singh U and Rao PV (1997): Effect of varieties and processing methods on the total and ionizable iron contents of grain legumes. *J.Agric.Food Chem*. 45: 3859 - 3862

Cornovale E, Lugaro E and Lombardi-Boccia G (1988): Phytic acid in faba bean and peas: effect on protein availability. *Cereal Chem*. 65: 114 – 117

Cossack ZT and Prasad AS (1983): Effect of protein source on the bioavailability of zinc in human subjects. *Nutr.Res*. 3: 23 -31

Davies NT, Hristic V and Flett A (1977): Phytate rather than fibre in bran as the major determinant of zinc availability to young rats. *Nutr.Rep.Int*. 15: 207 – 214

Davies NT, Carswell AJP and Mills CF (1984): The effect of variation in dietary calcium intake on the phytate- zinc interaction in rats. In: Proc. 5th. Int. Symp. Trace Element Metabolism in Man and Animals. Aberdeen, p125

Davies NT, Carswell AJP and Mills CF (1985): The effect of variation in dietary calcium intake on the phytate – zinc interaction in rats. In: Mills C F, Bremner I and Chesters J K, (Eds.): Trace Elements in Man and Animals. Farnham Royal, UK, Commonwealth Agricultural Bureau, pp. 456 -457

Ferguson EL, Gibson RS, Thompson, LU and Ounpuu S (1989): The dietary calcium, phytate, and zinc intakes, and the calcium, phytate, and zinc molar ratios of a selected group of East African children. *Am. J. Clin. Nutr* 50: 1450 – 1456

Forbes RM, Parker HM and Erdman JW, Jr. (1984): Effects of dietary phytate, calcium and magnesium levels on zinc bioaccessibility to rats. *J Nutr*. 114: 1421-1425

Gibson RS (1994): Content and bioaccessibility of trace elements in vegetarian diets. *Am. J Clin.Nutr*. 59 Suppl.: 1223S – 1232S

Gibson RS, Yeudall F, Drost N, Mtitimuni B and Cullinan T (1998): Dietary interventions to prevent zinc deficiency. *Am.J.Clin.Nutr*. 68 Suppl.: 481S - 487S.

Gilooly M, Bothwell TH, Torrance JD, MacPhail AP, Derman DP, Bezwoda WR, Mills W, Charlton RW and Mayet F (1983): The effect of organic acids, phytates, and polyphenols on the absorption of iron in vegetables. *Br.J.Nutr*. 49: 331-342

Glahn RP, Cheng Z, Welch RM and Gregorio GBA (2002): Comparison of iron bioavailability from 15 rice genotypes: Studies using an in vitro digestion / Caco-2 cell culture model. *J.Agric.Food Chem*. 50: 3586-3591

Glahn RP, Wortley GM, South PK and Miller DD (2002a): Inhibition of iron uptake by phytic acid, tannic acid and ZnCl: Studies using an *in vitro* digestion / Caco-2 cell model. *J.Agric.Food Chem*. 50: 390 – 395

Hallberg L, Brune M, and Rossander L (1989): Iron absorption in man: ascorbic acid and dose- dependant inhibition by phytate. *Am.J.Clin.Nutr*. 49: 140 -144

Hamdaoui M, Doghri T, and Tritar B. (1995): Effect of different concentrations of an ascorbic acid and tea mixture on non-heme iron absorption from a typical Tunisian meal fed to healthy rats. *Ann.Nutr.Metab*. 39: 310-316

James WPT (1980): Dietary fibre and mineral absorption. In: Medical Aspects of Dietary Fibre. Spiller GA and Kay RM (Eds):. Plen Medical Book Co. New York, pp 239 - 259

Jood S and Kapoor AC (1997): Improvement in bioavailability of minerals of chickpea and black gram cultivars through processing and cooking methods. *Int.J.Food Sci.Nutr.* 48: 307 - 312

Kaur M and Kawatra BL (2002): Effect of domestic processing on zinc bioavailability from rice bean (*Vigna umbellata*): diets. *Plant Foods Hum.Nutr.* 57: 307-318

Kelsay JL, Prather ES, Clark WM and Canary JJ (1988): Mineral balances of men fed a diet containing fiber in fruits and vegetables and oxalic acid in spinach for six weeks. *J.Nutr*. 118: 1197-204

Lombardi-Boccia G, De Santis N, Di Lullo G and Carnovale E (1995): Impact of processing on Fe dialysability from bean (*Phaseolus vulgaris* L.): *Food Chem.* 53: 191-195

Lonnerdal B, Sandberg AS, Sandstrom B and Kunz C (1989): Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J.Nutr*. 119: 211 – 214

Luten J, Crews H, Flynn A, Dael PV, Kastenmayer P, Hurrel R, Deelstra H, Shen LH, Fairweather-Tait S, Hickson K, Farre R, Schlemmer U and Frohlich W (1996): Inter-laboratory trial on the determination of the *in vitro* iron dialyzability from food. *J. Sci. Food Agric.* 72: 415-424

Navert B, Sandstrom B and Cederblad A (1985): Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. *Br.J.Nutr*. 53: 47 – 53

Oberleas D and Harland BF (1981): Phytate content of foods: effect on dietary zinc Bioaccessibility. *J. Am. Dietet. Assoc.* 79: 433-436

O'Dell BL (1984): Bioavailability of trace elements. *Nutr. Rev*. 42: 301-308

Prasad AS (2003): Zinc deficiency in humans: Effect on cell mediated immunity. In: Nutrition Goals for Asia – Vision 2020. First edition, Nutrition Foundation of India, New Delhi pp 349-358

Price ML, Scoyoc SV and Butler LG (1978): A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J.Agric.Food Chem*. 26: 1214-1218

Rossander-Hulten L, Sandberg AS and Sandstrom B (1992): The influence of dietary fibre on mineral absorption and utilization. In: Dietary Fibre – A Component of Food. Schweizer T and Edwards CA (Eds): Springer, Berlin, pp197 - 210

Sandberg AS (2002): Bioaccessibility of minerals in legumes. *Br.J.Nutr*. 88 (Suppl.3): S281 – S 285.

Sandberg AS, and Svanberg U (1991): Phytate hydrolysis by phytase in cereals; effects on *in vitro* estimation of iron availability. *J. Food Sci*. 56: 1330 - 33

Sandberg AS, Anderson H, Carlsson NG, and Sandstrom B (1986): Degradation products of bran phytate formed during digestion in the small intestine: effects of extrusion cooking on digestibility. *J.Nutr*. 117: 2061-2065

Sandberg AS, Rossander-Hulten L and Turk M (1996): Dietary *Aspergillus niger* phytase increases iron absorption in humans. *J.Nutr*. 126: 476-480

Sandberg AS, Brune M, Carlsson NG, Hallberg L, Skogland and Rossander-Hulten L (1999): Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *Am.J.Clin.Nutr*. 69: 240-246

Sandstrom B (1989): Dietary pattern and zinc supply. In: Zinc in Human Biology. Mills CF (Ed): Springer-Verlag, London; pp. 351 – 363

Schwartz R, Apgar BJ, and Wien EM (1986): Apparent absorption and retention of Calcium, Copper, Magnesium, Manganese, and Zinc from a diet containing bran. *Am.J.Clin.Nutr*. 43: 444-455

Sebastia V, Barbera R, Farre R & Lagarda MJ (2001): Effect of legume processing on calcium, iron and zinc contents and dialysabilities. *J.Sci.Food Agric.* 81: 1180-1185

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG, MacPhail P, Schmidt U, Tal A and Mayet F (1991): Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on non-heme iron absorption. *Am.J.Clin.Nutr*. 53: 537-541

Snedecor GW and Cochran WG (1977): In: Statistical Methods. Ames: Iowa State Univ. Press, p.100

Snedeker SM and Greger JL (1983): Effects of dietary protein, sulfur amino acids and phosphorus on human trace element metabolism. *J. Nutr*. 113: 644-652

Svanberg U and Sandberg AS (1988): Improve iron bioavailability in weaning foods through the use of germination and fermentation. In: Improving Young Child Feeding in Eastern Southern Africa: Household-Level Food Technologies. Alnwick D, Moses S and Schmidt OG (Eds.): International Development Research Centre, Nairobi, Kenya, pp.366-373

Thompson DBand Erdman JW Jr. (1982): Phytic acid determination in soybeans. *J.Food Sci*. 47: 513-517

Torre M, Rodriguez AR and Saura–Calixto F (1991): Effect of dietary fibre and phytic acid on mineral availability. *Crit.Rev.Food Sci.Nutr*. 30: 1-22

Troost FJ, Brummer RM, Dainty JR, Hoogewerffr JA, Bull VJ and Saris WHM (2003): Iron supplements inhibit zinc but not copper absorption *in vivo* in ileostomy subjects. *Am. J. Clin. Nutr*. 78: 1018-1023

Turk M and Sandberg AS (1992): Phytate degradation during bread making: Effect of phytase addition. *J.Cereal Sci.* 15: 281-294

Turnland JR, King JC, Keyes WR, Gong B and Michel MC (1984): A stable isotope study of zinc absorption in young men: effects of phytate and cellulose. *Am. J. Clin. Nutr*. 40: 1071 -1077

Tutanwiroon M, Sritongkul N, Brune M, Rossander– Hulten L, Pleehachinda R, Suwanik R and Hallberg L (1991): Dose-dependent inhibitory effect of phenolic compounds in foods on non haem absorption in men. *Am.J.Clin.Nutr*. 53: 554 – 557

Watzke HJ (1998): Impact of processing on bioavailability of minerals in food. *Trends Food Sci.Technol*. 9: 320-327

Wise A (1983): Dietary factors determining the biological activities of phytate. *Nutr.Abstr*. Rev. 53: 791-806

CHAPTER-III

Andrews NC (1999): The ion transporter DMT 1. *Int.J.Biochem.Cell Biol*. 31: 991 – 994

Davidsson L, Almgren A, Sandstrom B and Hurrell RF (1995): Zinc nutrition in adult humans: the effect of iron fortification. *Br.J.Nutr*. 74: 417 -425

Dawson EB, Albers J, and McGaniti WJ (1989): Serum zinc changes due to iron supplementation in teenage pregnancy. *Am.J.Clin.Nutr*. 50: 848 – 852

Dawson–Hughes B, Seligson FH and Hughes VA (1986): Effect of calcium carbonate and hyroxyapatite on zinc and iron retention in post-menopausal women. *Am.J.Clin.Nutr*. 44: 83 -88

Fairweather–Tait SJ, Wharf SG and Fox TE (1995): Zinc absorption in infants fed iron fortified weaning food. *Am.J.Clin.Nutr*. 62: 785 – 789

Gibson RS (1994): Content and bioavailability of trace elements in vegetarian diets. *Am J Clin Nutr* 59 Suppl: 1223S – 12232S

Gibson RS, Yeudall F, Drost N, Mtitinuni B and Cullinan T (1998): Dietary interventions to prevent zinc deficiency. *Am.J.Clin.Nutr*. 68 Suppl: 481S - 487S

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL and Hediger MA (1997): Cloning and characterization of a mammalian proton coupled metal – ion transporter. *Nature* 388: 482 – 488

Hurrell RF, Lynch TP, Dassenko SA and Cook JD (1989): Iron absorption in humans as influenced by bovine milk proteins. *Am.J.Clin.Nutr.* 49: 546 – 552

Keane AP and Miller BD (1984): In vitro estimation of the effects of selected proteins on iron bioavailability. *Am.J.Clin.Nutr.* 40: 393- 401

Lombardi-Boccia G, Carbonaro M, Di Lullo G and Carnovale E (1994): Influence of protein components on iron and zinc dialyzability from *Phaseolus vulgaris*. *Int.J.Food Sci.Nutr.* 45: 183 - 190

Luten J, Crews H, Flynn A, Dael PV, Kastenmayer P, Hurrel R, Deelstra H, Shen LH, Fairweather-Tait S, Hickson K, Farre R, Schlemmer U and Frohlich W (1996): Inter-laboratory trial on the determination of the *in vitro* iron dialyzability from food. *J. Sci. Food Agric.* 72: 415-424.

Nelson KJ and Potter NN (1980): Iron availability from wheat gluten, soy isolate, and casein complexes. *J.Food Sci.* 45: 52 – 56

Prasad AS, Brewer GJ, Schoomaker EB and Rabbani P (1978): Hypocupraemia induced by zinc therapy in adults. *J.Am.Med.Assoc.* 240: 2166 - 2168

Rossander–Hulten L, Brune M, Sandstrom B, Lonnerdal B and Hallberg L (1991): Competitive inhibition of iron absorption by manganese and zinc in humans. *Am.J.Clin.Nutr.* 54: 152 – 156

Sandstrom B and Cederblad A (1980): Zinc absorption from composite meals. II. Influence of the main protein source. *Am.J.Clin.Nutr.* 33: 1778 – 1783

Sandstrom B, Arvidsson B, Cederblad A and Bjorn-Rasmussen E (1980): Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc, calcium and protein content in meals based on bread. *Am.J.Clin.Nutr.*33: 739 - 745

Sandstrom B, Cederblad A and Lonnerdal B (1983): Zinc absorption from human milk, cow's milk and infant formulas. *Am. J. Dis. Child.* 137: 726 - 729

Sandstrom B, Davidsson L, Cederblad A and Lonnerdal B (1985): Oral iron, dietary ligands and zinc absorption. *J.Nutr.* 115: 411 – 414

Sandstrom B (1992): Dose dependence of zinc and manganese absorption in man. *Proc.Nutr.Soc.* 51: 211 – 218

Simmer K and Thompson RP (1985): Maternal zinc and intra-uterine growth retardation. *Clin.Sci.*68: 395 – 399

Snedecor GW and Cochran WG (1977): In: *Statistical Methods*. Ames: Iowa State Univ. Press, p.100

Solomons NW (1986): Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *J.Nutr.* 116: 927 – 935

Spencer H, Kramer L, Norris C and Osis D. (1984): Effect of calcium and phosphorous on zinc metabolism in man. *Am.J.Clin.Nutr.* 40: 1213 – 1218

Valberg LS, Flanagan PR, Brennan J and Chamberlain ML (1985): Effects of iron, tin and copper on zinc absorption in humans. *Am.J.Clin.Nutr.* 41: 536 - 541

Waggle DH, Steinke FH and Shen JL (1989): Isolated soy proteins. In: Legumes. Matthews RH (Ed.):, Marcel Dekker, New York, pp 99 - 138

CHAPTER-IV

Amoa B and Muller HG (1976): Studies on kenkey with particular reference to calcium and phytic acid. *Cereal. Chem.* 53: 365-375

Bartnick M & Szafranska I (1987): Change in phytate content and phytase activity during the germination of some cereals. *J.Cereal Sci.* 5: 23 - 28

Camacho L, Sierra C, Campos R, Guzman E and Marcus D (1992): Nutritional changes caused by germination of legumes commonly eaten in Chile. *Arch. Latin Americanos De Nutricion.* 42: 283 - 290

Chang R, Schwimmer S and Burr HK (1977): Phytate: removal from whole dry beans by enzymatic hydrolysis and diffusion. *J. Food Sci.* 42:1098-1101

Chavan JK and Kadam SS (1989): Nutritional improvement of cereals by fermentation. *Crit. Rev. Food Sci. Nutr.* 28: 349-400

Chopra S and Sankhala A (2004): Effect of soaking and sprouting on tannin, phytate and *in vitro* iron in under-utilized legumes - Horse gram (*Dolichos biflorus*): and Moth bean (*Phaseolus aconitifolius*): *J. Food Sci. Tech.* 41: 547-550

De Maeyer BM, Dallman P, Gurncy JM, Hallberg L, Sood SK and Srikantia SG (1989): Preventing and controlling iron deficiency anaemia through primary health care – A guide for health administrators and programme managers. WHO, Geneva

Devadas RP (1998): Local strategies to support child nutrition. *Nutr. Res.* 18: 233-239

Drago SR and Valencia ME (2002): Effect of fermentation on iron, zinc and calcium availability from iron fortified dairy products. *J. Food Sci.* 67: 3130-3134.

Duhan A, Khetrapaul N and Bishnoi S (2004): HCl - extractability of zinc and copper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *J. Food Comp. Anal.* 17: 597-604

Ene-Obong HN and Obizoba IC (1996): Effect of domestic processing on the cooking time, nutrients, antinutrients and *in vivo* protein digestibility of the African yam bean. *Plant Foods Hum. Nutr.* 49: 43-52

Gibson RS (1994): Content and bioavailability of trace elements in vegetarian diets. *Am. J Clin. Nutr.* 59 Suppl.: 1223S-1232S

Gibson RS and Hotz C (2001): Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *Br. J. Nutr.* 85 Suppl. : S159-S166

Hamdaoui M, Doghri T and Tritar B (1995): Effect of different concentrations of ascorbic acid, tea mixture on non-haem iron absorption from a typical Tunisian meal fed to healthy rats. *Ann. Nutr. Metab.* 39: 310-316

Hirabayashi M, Matsui T & Yano H. (1998): Fermentation of soybean meal with *Aspergillus usamii* improves zinc availability in rats. *Biol.Trace Element Res.* 61: 227 -234

Hotz C and Gibson RS (2001): Assessment of home - based processing methods to reduce the phytate content and phytate: zinc molar ratio of white maize. *J. Agric. Food Chem.* 49: 692-698

Kannan S, Nielson SS, Patricia A, Rodriguez–Burger and Mason AC (2001): Iron and zinc bioavailability in rats fed intrinsically labeled bean and bean - rice infant weaning food products. *J. Agric. Food Chem.* 49: 5063-5069

Kaur M and Kawatra BL (2002): Effect of domestic processing on zinc availability from rice bean (*Vigna umbellata*) diets. *Pant. Foods Hum. Nutr*. 57: 307-318

Lestienne I, Icard-Verniere C, Mouquet C, Picq C and Treche S (2005): Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* 89: 421-425

Lombardi-Boccia G, De Santis N, Di Lullo G and Carnovale E (1995): Impact of processing on Fe dialysability from bean (*Phaseolus vulgaris* L.). *Food Chem.* 53: 191-195

Lorenz K (1980): cereal sprouts: Composition, nutritive value, food applications. *Crit. Rev. Food Sci. Nutr.* 13: 353-385

Luten J, Crews H, Flynn A, Dael PV, Kastenmayer P, Hurrel R, Deelstra H, Shen LH, Fairweather-Tait S, Hickson K, Farre R, Schlemmer U and Frohlich W (1996): Inter-laboratory trial on the determination of the *in vitro* iron dialyzability from food. *J. Sci. Food Agric.* 72: 415-424

Oberleas D and Harland BF (1981): Phytate content of foods: Effect on dietary zinc bioavailability. *J. Am. Dietet. Assoc.* 79: 433-436

Prabhavathi T and Rao, BSN (1979): Effects of domestic preparation of cereals and legumes on ionizable iron. *J. Sci. Food. Agric.* 30: 597-602

Price ML, Scoyoc SV and Butler LG (1978): A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* 26: 1214-1228

Reddy NR, Pierson MD, Sathe SK and Salunke DK (1989): Phytates in cereals and legumes. CRC Press, Boca Raton, pp. 68-72

Sandberg AS (1991): The effect of food processing on phytate hydrolysis and availability of iron and zinc. In: Nutritional and Toxicological Consequences of Food Processing. Friedman M (Ed): Plenum Press, New York, pp. 499-508

Sandberg AS and Svanberg U (1991): Phytate hydrolysis by phytase in cereals; effects on *in vitro* estimation of iron availability. *J. Food Sci.* 56: 1330-1333

Sandberg AS (2002): Bioavailability of minerals in legumes. *Br. J. Nutr.* 88 Suppl: S281-S 285

Snedecor GW and Cochran WG (1977): Statistical Methods. Iowa State Univ. Press, Ames

Svanberg U, Lorri W and Sandberg AS (1993): Lactic fermentation of non-tannin and high tannin cereals: Effects on *in vitro* estimation of iron availability and phytate hydrolysis. *J. Food Sci.* 58: 408-412

Tangkongchitr U, Seib PA and Hoseney RC (1982): Two barriers to the loss of phytate during bread making. *Cereal Chem.* 59: 216-221

Thompson DB and Erdman JW (1982): Phytic acid determination in soybeans. *J. Food Sci.* 47: 513-517

Tontisirin K, Mantel G and Battacharjee L (2002): Food based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proc. Nutr. Soc.* 61: 243-250

Turnland JR, King JC, Keyes WR, Gong B and Michel MC (1984): A stable isotope study of zinc absorption in young men: Effects of phytate and cellulose. *Am. J. Clin. Nutr.* 40: 1071-1077

CHAPTER-V

Gibson RS (1994): Content and bioavailability of trace elements in vegetarian diets. *Am. J.Clin.Nutr*. 59 Suppl.: 1223S - 1232S

Gillooly M, Bothwell TH, Torrance JD, MacPhail AP, Derman DP, Bezwoda WR, Mills W, Charlton RW, (1983): The effect of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br.J.Nutr*. *49:* 331-342

Lonnerdal B (2002): Phytic acid - trace element (Zn, Cu, Mn): interactions. *Int.J.Food Sci.Technol.* 37: 749-758

Luten J, Crews H, Flynn A, Dael PV, Kastenmayer P, Hurrel R, Deelstra H, Shen LH, Fairweather-Tait S, Hickson K, Farre R, Schlemmer U and Frohlich W (1996): Inter-laboratory trial on the determination of the *in vitro* iron dialyzability from food. *J. Sci. Food Agric.* 72: 415-424

National Institute of Nutrition (1998): Dietary Guidelines for Indians- A Manual. Indian Council of Medical Research, Hyderabad, p 75

O'Dell BL (1984): Bioavailability of trace elements. *Nutr.Rev.* 42: 301-308

Price ML, Scoyoc SV, and Butler LG (1978): A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J.Agric.Food Chem*. 26: 1214-1218

Sandberg AS (2002): Bioavailability of minerals in legumes. *Br.J.Nutr*. 88 Suppl.: S281-S285

Snedecor GW and Cochran WG (1977): *Statistical Methods*. Iowa State Univ.Press, Ames p.100