NFI BULLETIN
Bulletin of the Nutrition Foundation of India
Volume 35 Number 4
October 2014

Addressing Iron deficiency through Biofortification

Ajay Parida and S. Rajalakshmi

Among many of the global concerns that face Mankind today, micronutrient malnutrition is considered a priority, requiring urgent and concerted attention. Micronutrient deficiency, with long-ranging effects on health, learning ability and productivity, contributes to the vicious cycle of malnutrition, underdevelopment and poverty. In India, malnutrition is particularly prevalent among women, children and adolescents, with almost 50% of children under 5 being reported to be underweight (weight for age) and stunted (height for age). The prevalence of undernourishment among adults in the country is reported to be over 30%.

Among the micronutrient deficiencies (Figure 1), iron deficiency anaemia (IDA) is the most serious public health problem affecting over two billion people (or about one-third of the world's population). According to WHO mortality data, around 0.8 million deaths (1.5% of the total) can be attributed to iron deficiency each year. Estimates of IDA in women and children vary between 50-70%, pregnant women being particularly susceptible[1]. Its most visible impact is iron deficiency anaemia (IDA) which contributes significantly to the high levels of maternal and neonatal deaths in poor, vulnerable populations; however, the 'hidden' impact of iron deficiency extends to all areas of individual growth and development. The insidious nature of IDA has made it a difficult challenge for the international community to address effectively, as its scale and impact are often overlooked.

Among various strategies adopted globally, food-based approaches that include food production, dietary diversification and food fortification are considered to be sustainable strategies for improving the micronutrient status of populations and raising the levels of nutrition. Food-based approaches focus on practical, sustainable action through increasing availability, access to, and consumption of adequate quantities and appropriate varieties of safe and good quality food.

Biofortification

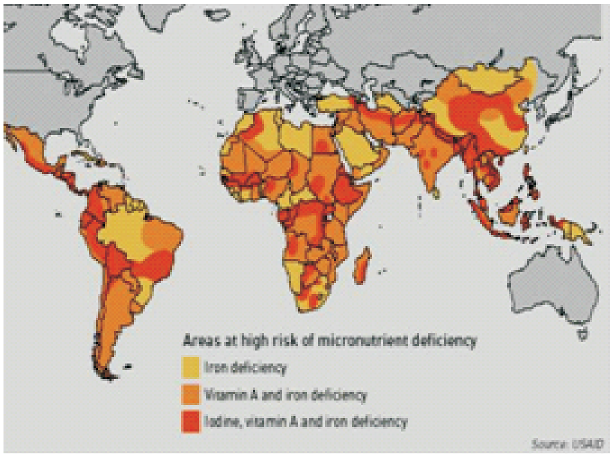
Biofortification involves the use of traditional crop breeding practices or modern biotechnology to increase the micronutrient concentration in crops. This type of intervention aims at addressing the specific micronutrient deficiencies of a target population[2,3]. Early studies have demonstrated some success. Mozambique promoted the production, access to, and consumption of biofortified orange-fleshed sweet potatoes (OFSP) to tackle vitamin A deficiency in young children. Farmers' access to improved orange-fleshed sweet potato vines and roots increased, nutrition knowledge improved, demand for this product increased, and a stable market for the vegetable was established. Subsequently, Vitamin A intake was shown to have increased due to increased OFSP intake, and serum retinol concentrations showed improvement in young children in the rural communities[4]. Similarly, Nirmal seeds in collaboration with HarvestPlus Program of the Consultative Group on International Agricultural Research (CGIAR) has developed and deployed iron rich pearl millet hybrids. Developed using germplasm screening and plant breeding approach, this millet variety (ICTP-8203) has 50-65 ppm iron, about double that in modern wheat varieties and with 10-15% higher yield (Figure 2). It is clear, therefore, that the biofortification approach complements other interventions and is a viable means to provide micronutrients to the most vulnerable sections of the population in a comparatively inexpensive and cost-effective way, using sustainable agricultural interventions[5].

Rice biofortification to enhance iron content

An Indian Network on Biofortification was launched in 2005 with support from the Department of Biotechnology, Government of India, for the purpose of developing micronutrient-rich cultivars in three staple crops, rice, wheat and maize. The network programme on "Rice biofortification with enhanced iron and zinc in high yielding non-basmati rice cultivars through marker assisted breeding and transgenic approaches" is coordinated by M. S. Swaminathan Research Foundation and has Tamil Nadu Agriculture University, Coimbatore, University of Agriculture Science, Bangalore, Indira

CONTENTS

Figure 1: Worldwide micronutrient deficiences



Gandhi Agriculture University, Raipur and Directorate of Rice Research as partners.

Rice has good potential for nutrient fortification because it has the following natural advantages: (i) it is a major staple food that is consumed by almost half of the world's population; (ii) in comparison with other plant foods it is a primary source not only of calories but also of protein; (iii) rice proteins have very low allergenicity and the crop does not have any toxic substances; and (iv) a very high level of expression of the desired traits can be obtained in this crop.

In rice, most of the iron is stored in the outermost layer known as the aleurone layer. Therefore, during commercial milling/polishing, most of the iron in the rice grains gets eliminated completely[6]. The nutrients are drastically reduced (to about one-third) during milling. Welch and Graham[7], showed that normal brown rice has 16 µg/g dry weight of iron, but after milling it gets reduced to 5 µg/g dry weight (90% extraction).

The approaches followed for rice biofortification are (i) to screen the natural germplasm resources (varieties, landraces, breeding lines) from the rice growing areas, (ii) carry out marker-assisted breeding and development of mapping populations; and (iii) employ transgenic and genomics tools for greater accumulation, transport and bioavailability of the micronutrient.

Figure 3: Range and Variability in Grain Micronutrient in selected Rice Genotypes

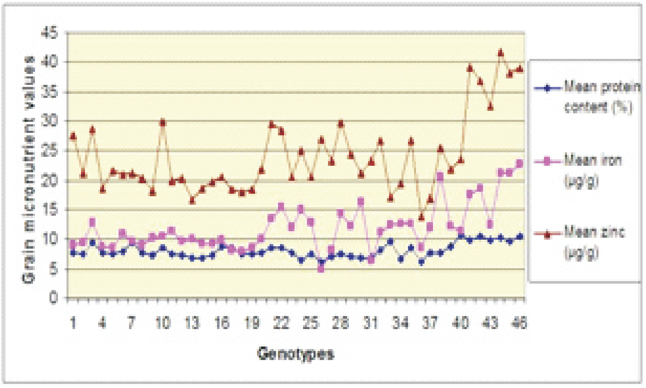


Figure 2: High-iron commercial cultivars of pearl millet
ICTP 8203; 70-74 ppm Fe: With 10% Higher Yield

Germplasm screening and identification of Rich Micronutrient lines

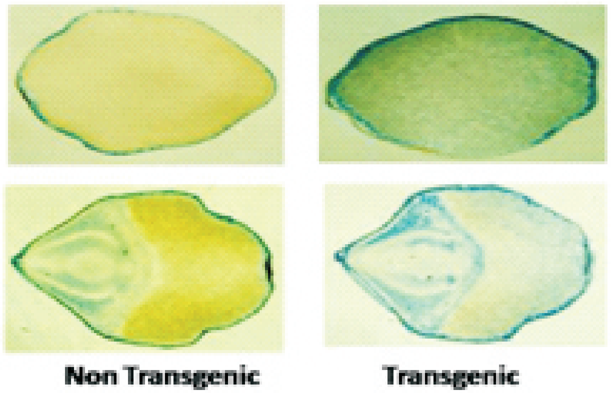
Procedures for precise estimation of iron and zinc concentrations in grains were standardized using an atomic absorption spectrometer. More than 3500 rice accessions, including ~252 rice landraces of CG collection known for high grain nutritive value, ~100 popular rice cultivars and ~650 advanced breeding lines developed through various breeding populations have been screened. Low genetic variations were observed for grain Fe levels as compared to grain Zn content. The 160 landraces from Odisha, Tamil Nadu and Kerala analysed at MSSRF had 12 accessions with high iron content that ranged from 12-18ppm in unpolished rice.

Grain micronutrient concentration is a polygenic trait showing complex inheritance and has been reported to be affected by environmental factors such as native soil properties (saline, sodic, organic matter content), external application of nitrogen (N), phosphorus (P), potassium (K) fertilizer or micronutrients, and occurrence of abiotic stresses during the growing period, such as drought, water-logging etc. Multi-location agronomic trials have been conducted for selected cultivars high in iron and zinc, screened in order to identify stable and location-specific genotypes and their response to both foliar and soil supplementation with zinc and iron. The variations observed in grain Fe/Zn content in rice under different soil conditions suggested that the differential response of genotypes as regards Fe/Zn uptake and translocation is the major limiting factor influencing the accumulation of these micronutrients in the grains under certain soil conditions. The efficiency of a genotype for N or Fe/Zn uptake, translocation, assimilation and/or redistribution was found to be highly heritable, and external soil and fertilizer conditions influence these biological processes to variable extents Figure 3).

Marker-assisted breeding

The polygenic complex nature of inheritance as regards grain nutritive traits necessitates the use of a molecular marker assisted breeding (MAB) approach to develop nutritionally rich rice. Simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs) are the markers of choice for most plant breeders. The availability of the complete genome sequence of japonica and indica rice subspecies has paved the way for computational genomic studies to discover new markers as well as to provide the basic framework for experiments involving expression analysis of candidate genes. The technical simplicity and reliability of the procedure as well as the high number of polymorphic loci have

Figure 4: Tissue specific expression of Ferritin in grains



increased the applicability of genomic SSRs, EST-SSRs and SNP markers in MAB. Microsatellite markers close to candidate genes for grain iron concentration are used for genotyping of populations derived from crosses, to identify quantitative trait loci (QTLs) for grain micronutrient content. The genotyping of the mapping populations are carried out for candidate genes based on SSR and SNP based DNA markers. Many research groups have reported encouraging leads in using the marker assisted breeding approach. Chandel et al[8] identified four metal-related homeostasis candidate genes for the development of DNA markers in iron / zinc homeostasis, showing good correlation between gene expression data (based on semi quantitative RT- PCR analysis) and actual grain Fe and Zn content . In MSSRF, 160 landraces from Odisha, Tamil Nadu and Kerala were screened for high iron content. The iron content ranged from 35 - 49 ppm in unpolished rice. When these landraces are subjected to SSR marker analyses, it will lead to the development of enriched iron / zinc lines. Several mapping populations using high iron enriched donors and recipient popular varieties are being developed at Various centres for identifying new QTLs linked to iron accumulation in grains, which can then be backcrossed for developing useful biofortified varieties.

Transgenic and genomic approach to biofortification

Molecular breeding and functional genomics approach are useful tools for enhancing micronutrient accumulation in staple crops. It has been well established that grain protein and Fe/Zn values are polygenic traits, requiring to be explored at molecular levels. A good understanding of the molecular biology underlying grain protein accumulation and the factors that affect the rate of Fe/Zn absorption, transport and remobilization into grains will provide valuable information regarding inheritance patterns and will help in designing breeding strategies to develop rice varieties with high nutritive value without compromising yield.

Figure 6: Grain iron content in transgenic lines

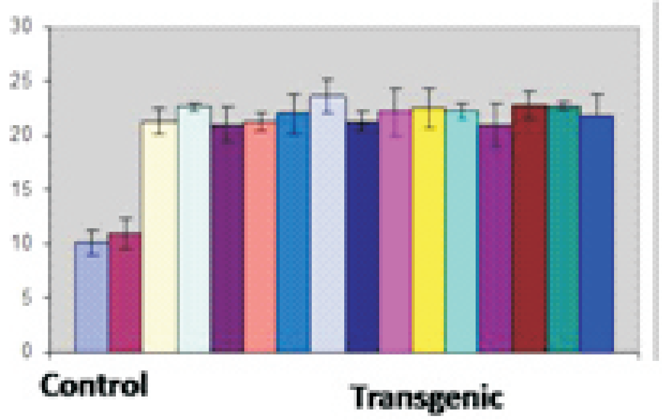
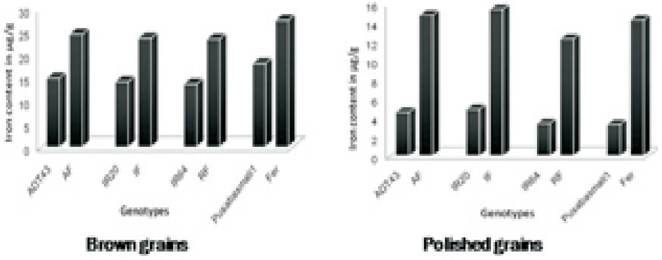


Figure 5: Iron content in the grains of AmFer1 introgressed lines along with Recurrent and Donor parents



Recent studies clearly demonstrate that overexpression of ferritin in transgenic rice plants resulted in two-to three-fold increases in iron content in seeds (Figure 4). This implies that the low iron concentration in the seed may not result from low iron availability for transport, but rather, from lack of iron-sequestering capacity in the seeds. Therefore, increasing the sequestering capacity of seeds by overexpressing the ferritin gene in indica rice cultivars will increase the iron content, thereby serving the nutritional needs of vulnerable populations in India.

Using large scale EST sequencing approach, 1800 ESTs were generated from the mangrove species Avicennia marina cDNA library. The EST-sequencing analysis revealed the presence of a number of ferritin cDNA clones (ESTs) in the A. marina cDNA library, with potential for developing transgenic rice varieties with enhanced iron content. A full-length ferritin gene (AmFer1) was isolated from A. marina using TAIL PCR and RT-PCR methods (Figure 5). The basic function of ferritin is to take up iron, store it in a non-toxic form, and release it for metabolic functions strictly on need basis[9,10].

The iron content in the transgenic plants (seeds) was analysed at T1 segregating population to examine the effect of the introduced ferritin gene (Figure 6). The iron content in the matured transgenic seeds from line 12 varied from 20.9±1.2 to 22.6±2.2 µg/g, twice as high as that in non-transformed control seeds (8.9±2 µg/g to 10.9 ± 1.6 µg/g).

Backcross breeding

Backcross breeding is commonly employed to transfer one or more genes from a donor variety which is poor in other agronomic characters to an elite variety. It is the final step in the development of transgenic events in the background of elite local varieties. Given that not all the cultivars are innately amenable to transformation, transgenic events are developed and characterized in the cultivars that have high levels of transformation efficiency, and are introgressed to the elite varieties by backcross breeding[11]. The conventional approach to backcross breeding requires five to six generations of backcrosses to develop an isogenic line, whereas marker-assisted backcrossing can speed up the process of recovery, making isogenic lines available even at two or three backcross generations[12]. Using the backcross breeding approach, introgression of the selected enhanced iron lines was carried out in four popular Indica rice varieties (IR 64, ADT 43, Pooni and IR 20). In successive generations each of these introgressed lines has shown a more than two-fold increase in Fe content in the polished grains.

Currently research efforts are focused on enhancing the transportation of iron to the grains. A cis-acting element binding factor 1 (PcIDEF1) encoding for an iron deficiency responsive gene has been identified and isolated from Porteresia coarctata, a wild relative of rice. In rice, the homologue of this gene plays an important role in regulating iron-deficiency-inducible genes involved in maintaining iron homeostasis. Similarly, three metal-related candidate genes NAS 2, Ferritin and FRO2 with high iron content have been identified in barnyard millet, and these will be utilized for enhancing the micronutrient content in Rice.

Biofortification is only one of the approaches to addressing micronutrient deficiency. It is complementary to several other approaches being practiced and advocated. It is therefore essential to develop a unified approach by promoting science-based farming to enhance the nutritive value of harvested food grains and also creating greater awareness so that rural households can meet their nutritional requirements from local sources. It would be beneficial to follow a Farming System for Nutrition (FSN) approach. This approach is being conceptualized and field tested by the M. S. Swaminathan Research Foundation. Prof. M. S. Swaminathan says "FSN involves the introduction of agricultural remedies to the nutritional maladies prevailing in an area, through the mainstreaming of nutritional criteria in the selection of the components of a farming system involving crops, farm animals and, where feasible, fish. While finalizing the components of a farming system, the gender and age dimensions of human nutritional needs should be kept in view, such as the special needs of pregnant women and nursing mothers, and newborn babies during the first 1000 days after conception and birth."

The authors are from M. S. Swaminathan Research Foundation, Chennai 600112, India. (e-mail: ajay@mssrf.res.in)

References

1. www.who.int/ nutrition/publications/micronutrients.
2. Kennedy G, Pedro MR, Seghieri C, Nantel G, Brouwer I. Dietary diversity score is a useful indicator of micronutrient intake in non breast-feeding Filipino children J. Nutr. ;137:1–6, 2007.
3. Bouis H, Welch RM. Biofortification - A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South Crop Sci; 50:S20–S32, 2010.
4. Low J, Arimond M, Osman N, Cunguara B, Zano F, Tschirley D. A Food-Based Approach Introducing Orange-Fleshed Sweet Potatoes Increased Vitamin A Intake and Serum Retinol Concentrations in Young Children in Rural Mozambique J. Nutr;137:1320–1327, 2007.
5. Pfeiffer WH, McClafferty B. HarvestPlus: Breeding crops for better nutrition. Crop Sci. 2007. 47:S88, 2007.
6. Krishnan S, Datta K, Baisakh N, de Vasconcelos M, Datta SK. Tissue-specific localization of β-carotene and iron in transgenic indica rice (Oryza sativa L.) Current Science. 84, 1232-1234, (2003).
7. Welch RM ,Graham RD .A new paradigm for world agriculture : meeting human need -Productive ,Sustainable ,Nutritious .Fi Crops Res .60 1 -10 ,1999 .
8. Chandel G , Banerjee S , Vasconcelos M , Grusak MA . Characterization of the Root Transcriptome for Iron and Zinc Homeostasis -related Genes in Indica rice (Oryza sativa L) Journal of Plant Biochemistry and Biotechnology19 :145 -152 ,(2010) .
9. Seckbach J .Ferreting out the secrets of plant ferritin -a review .J Plant Nutr .5 :369 -394 ,1982 .
10. Briat JF .Role of Ferritin in plants .J .Plant Nutr .19 :1331 -1342 , 1996 .
11. Takeda S , Matsuoka M . Genetic approaches to crop improvement : responding to environmental and population changes Nature Review Genetics ,9 ,444 -457 ,(2008) .
12. Neeraja CN ,Maghirang -Rodriguez R ,Pamplona A ,Heuer S , Collard BCY ,Septiningsih EM ,et al .A marker -assisted backcross approach for developing submergence -tolerant rice cultivars Theoretical and Applied Genetics ,115 ,767 -776 ,2007 .

FOUNDATION NEWS

- With profound sorrow we announce the passing away of the respected paediatrician, Dr.Shanti Ghosh on 16th August 2014. Besides being a valuable member of the Governing Body of NFI, she was our friend and well-wisher. On behalf of the institution and its staff, Dr.C.Gopalan, President NFI and Dr. Prema Ramachandran, Director, wish to express their heartfelt condolence to Dr. Ghosh's family. We all will miss her.

- On 16th September 2014, a special Study Circle Lecture was organised jointly by NFI and NSI (Delhi Chapter) at the premises of NFI as a tribute to Dr. Shanti Ghosh. On this occasion, Dr. S.K. Bharghava and Dr. H.P.S. Sachdev spoke on "The New Delhi Cohort: Lessons Learnt", a widely cited research study initiated by Dr. Shanti Ghosh.

- Annual Foundation Day and C. Ramachandran Memorial Lecture: The Annual Foundation Day of NFI will be celebrated on 27th November 2014. On this occasion, Dr. V.M. Katoch, Secretary, Department of Health Research and Director General ICMR will deliver the C. Ramachandran Memorial Lecture.

NUTRITION NEWS

The 46th Annual Conference of the Nutrition Society of India will be held at Dayanand Medical College and Hospital, Ludhiana (Punjab) on 7-8 November, 2014. There will be a pre-conference workshop on 6th November 2014. The theme of the conference is 'Nutritional Approach for Combating Non Communicable Diseases in India'. The 38th Gopalan Oration will be delivered by Prof. Michael S. Kramer Dept. of Paediatrics and Epidemiology, McGill University, Montreal, Canada.

The 26th Dr. Srikantia Memorial Lecture will be delivered by Dr. Anura V. Kurpad, Prof. & Head, Division of Nutrition, St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore. The 5th Dr. Rajjamal P. Devadas Memorial Lecture will be delivered by Prof. G. Subbulakshmi Retired Director, PG Dept. &HOD FSN Dept. SNDT Women's University.

Dr Prema Ramachandran, Director Nutrition Foundation of India, New Delhi. will receive the Dr . B.K. Anand Memorial Award.

A symposium on "New Nutritional concepts in Foetal Programming" has also been organised during the meeting.
Details of the programme can be accessed on the Website: www.nsicon2014.org

Enhancing the bioavailability of natural and synthetic fortificants from foods through alteration in compositional matrices

Jamuna Prakash

Micronutrient malnutrition or 'hidden hunger' is a very well recognized public health problem in India. It is present across all age groups, leading to huge losses in terms of compromise of cognitive performance, increase in disability-adjusted life years (DALYs) and lowering of productive potential. Indian diets are deficient in many micronutrients, and sometimes even in macronutrients, as is evident from many nationwide dietary surveys. In terms of food production, India is self-sufficient in many respects. There was a remarkable transition in agricultural production due to the Green revolution. However, while the production of wheat and rice increased considerably, the production of other food items like pulses and coarse grains either remained stationary or declined. Lately, there has also been a substantial rise in the production of milk, fruits and vegetables, oilseeds, eggs, meat and marine products. Concurrently, the purchasing power has also shown improvement over the years as evidenced by income profiles of various populations. In addition, there are numerous government programmes to ensure availability of food to marginalized sections. As regards availability and accessibility of food, the situation in India can be described as 'food secure'; however, affordability may not exist universally.

The dietary patterns have changed from indigenous staple grains or starchy roots, locally grown legumes, vegetables and fruits and some animal foods to include more processed foods, refined grains, foods with higher fat, sugar or alcohol content and more foods of animal origin. The diets have become less nutritious and more energy-dense. While there has been a rise in quantity of food available, the dietary quality has not shown a concurrent improvement. The prevalence of malnutrition across all sections of the population is still high, and there are cases of clinical and sub-clinical deficiencies. Fifty six percent of under-five children are stunted, the incidence of low birth weight (>30%) is very high, and nearly 50% of pregnant women are anaemic. It is therefore clear that nutrition security is not automatically ensured merely by addressing food security.

The need for foods with enhanced nutrients

The adverse effects of micronutrient deficiencies are well known. It is also known that the existing Indian dietary patterns do not ensure adequate intake of all essential minerals and vitamins; however, this situation cannot be totally corrected or prevented even by increasing the intake of protective foods such as milk, fruits and vegetables. The reasons for this are: (i) the inherent low levels of nutrients in the food; (ii) lower levels of nutrients in the soil due to depletion over the years on account of using chemical fertilizers; and (iii) low bioavailability of minerals in Indian diets due to the presence of inhibiting factors, along with many other non-nutritional causes. Poor absorption of nutrients is one of the important concerns both in normal and malnourished individuals as well as in certain physiological disorders.

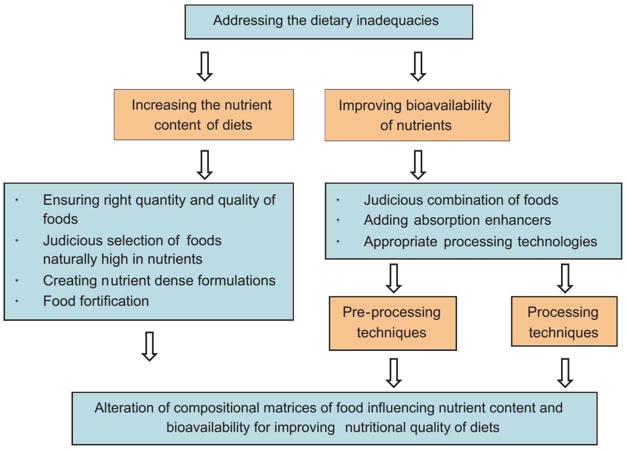
Adequacy of nutrients in the daily diet can be ensured by proper selection and judicious combination of foods to ensure the optimum availability and absorption of nutrients. While balanced diets do help in achieving adequate nutritional status, they are difficult to follow consistently. By effecting small modifications in day-to-day diets while selecting foods and by utilizing available technologies, it is possible to improve the micronutrient content in diets. The level of a given nutrient in a product can be enhanced by adding a chemical fortificant or by adding a nutrient-dense ingredient. The former can be an approach for public health intervention, whereas the latter is more suited for small-scale operations. This also gives scope for exploring alternative sources of nutrients such as underutilized materials, by-products of the food industry, and novel food sources. Both types of fortification will, of course, be subject to the effects of processing techniques which could alter the food matrix.

Fortification of foods, particularly staples, is one of the proven long-term strategies to counter hidden hunger. The benefits of large-scale fortification are well established: wide accessibility, easy implementation, and comparatively low cost. Hence, this is widely practiced in many nations. The science behind fortification is continuously evolving. The key elements are the fortificants and the vehicles, and their quantity and efficacy. The efficacy of a fortificant depends upon its absorption from the gut, which in turn is influenced by many host- and food-related factors, an important one among them being the food matrix. The nature of the fortificant and its bioavailability in the food chosen as a vehicle for addition are the influencing factors. The absorption of a nutrient from a soluble fortificant is generally more than from an insoluble fortificant. Apart from this, the presence of inhibiting and enhancing factors also influence both absorption and bioavailability.

This review examines two issues related to food fortification

- enhancing the content of micronutrients in the diet through proper selection of food, and
- increasing the bioavailability of nutrients by using appropriate technologies (based on selected studies from author's laboratory). The components comprising each issue in relation to altered compositional matrix of food are depicted in a conceptual framework in Fig 1.

Figure 1. IMPROVING NUTRITION THROUGH DIETARY APPROACHES



Food matrix and nutrient availability

Food matrix is the microstructure of food in which the nutrients and other components are embedded. It can be natural, altered or modified, or synthetic[1]. The food matrix has a large influence on the digestion and utilization (or physiological bioaccessibility) of nutrients at the gut level. Two important parameters that have an impact on the bioavailability of nutrients are the composition of the food and the processing techniques. The food matrix itself is dependent partly on the inherent composition of foods, and may be altered during processing.

The bioavailability of food is the fraction of an ingested nutrient that is available to the body for utilization in normal physiological functions or for storage. The factors that can affect bioavailability and digestibility of nutrients are: (i) the chemical state of the nutrient; (ii) the surrounding food matrix; (iii) the presence of and interaction with enhancing and inhibiting components; (iv) the nutrient density of the meal; and (v) the absorptive capacity of gastrointestinal tract. The food matrix affects the nutrient availability and digestibility by altering the gut environment. A considerable amount of data is available on the composition of foods and the beneficial and detrimental health effects of specific nutrients present in foods. However, the extent of availability for absorption in the gut is quite uncertain and varies for the same food depending on processing conditions, presence of other components, and so on[2,3].

The changing dietary pattern implies changes in the food matrix. Over the years, the food matrix has changed from totally unprocessed natural produce such as fruits, nuts, meat, cereal grains, vegetables, fruits, oilseeds, and milk to partially refined, semi-processed foods, and then to refined, processed, convenience foods. The extent to which foods are processed has been continually increasing. Some day in the future we may have highly processed designer foods prepared with isolated extracted components!

Natural food components are affected by processing. Macronutrients (carbohydrate, protein and fat) are not lost during processing, but it is possible that their properties and functionality are changed. Gelatinization of starch and denaturation of proteins are very common changes that occur during thermal processing. As regards micronutrients, minerals are usually stable but many vitamins and most of the bioactive components are lost during various kinds of processing[4].

Table 1. Chemical composition and mineral bioavailability from selected dehydrated green leafy vegetables (per 100g)

Green leafy vegetables	Total carotene (mg)	ß-carotene (mg)	Iron (mg)		Calcium (mg)		Dietary fiber (g)	
			Total	Bio-available	Total	Bio-available	Insoluble	Soluble
Amaranthus gangeticus	132943	26342	161.87	4.33	1651	101	31.33	4.20
Chenopodium album	138648	7068	23.33	1.98	513	89.3	19.95	6.39
Amaranthus sp.	187599	27950	36.46	1.87	1719	53.3	26.95	4.14
Peucedanum graveolens	175573	26867	46.67	2.37	656	266.5	24.96	3.93
Celosia argentea	113773	22267	125.59	5.03	1326	136.9	22.82	6.25
Centella asiatica	193890	25300	97.49	3.51	1248	405.8	29.05	3.52
Amaranthus tricolor	181343	28574	138.86	15.36	2065	81.2	23.21	5.28
Murraya koenigii	128985	17315	27.71	1.67	1488	320.6	39.08	2.90
Trigonella foenum graecum	129941	22286	33.54	0.38	1210	330.7	21.34	1.18

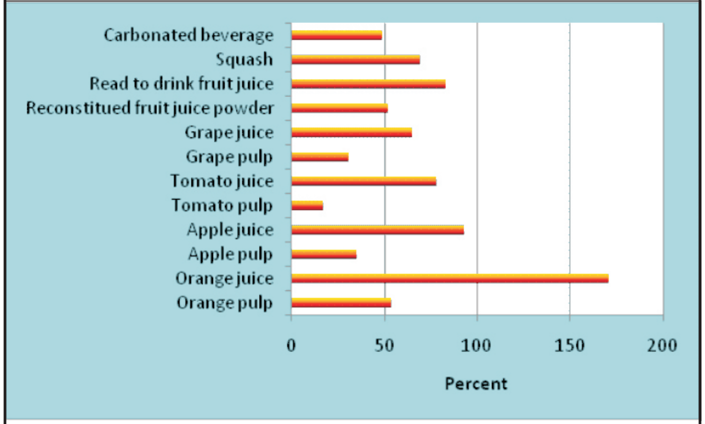
Note: Based on original paper, data source from the doctoral research of first author[5].

Different food processing operations can affect the food matrix in differing ways. Pre-processing operations such as sieving, polishing, and washing can result in a partial loss of nutrients; however, in some cases, these operations can actually increase the availability of the nutrients. The same is true for bioactive components. The natural food synergy is disturbed by refining processes such as polishing of rice, refining of wheat, extraction of oil from oilseeds, refining of oil, manufacturing sugar, hydrogenation of fat (giving rise to trans fat), etc. On the other hand, processing may result in favourable food synergy. For instance, carotenoids and xanthophylls require the presence of fat for absorption, lycopene is better absorbed in a ground form, and folate is better absorbed in free form than in the food matrix. The food matrix should be utilized optimally to enhance the bioavailability of essential components. This is applicable to natural components as well as added ones such as nutrients, bioactive components, nutrient extracts, beneficial microorganisms, fortificants, and isolated bioactive molecules.

Utilizing nutrient-dense ingredients

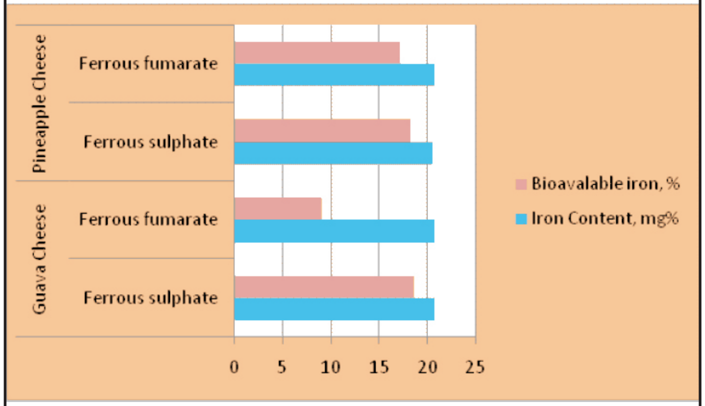
Green leafy vegetables are rich sources of calcium, iron, β-carotene, Vitamin C, dietary fiber and many trace minerals. A large variety of green leafy vegetable are available throughout the year in different parts of India. Such locally grown vegetables can be an economically viable source to ensure micronutrient intake. However, because they are often grown in waste water, their use entails considerable pre-preparation time for cleaning and cutting. Therefore, it is often impractical for working women to cook greens regularly even if they would like to do so. Their high water content renders these vegetables highly perishable in the absence of proper storage and transport facilities, and the lack of processing facilities at the production point. Dehydration is an appropriate technology to convert fresh greens to dry greens so that they can be stored for a longer time and used when needed. Drying reduces bulk, thereby facilitating packing and transportation. Dehydration results in some loss of nutrients, although minerals are largely retained. The nutrient losses can be compensated to some extent by concentration of nutrients in the dry greens. Table 1 shows that greens in dehydrated form can be a rich source of nutrients and dietary fibre[5]. Gupta et al. used multiple regression analysis to study the influence of various inhibitory factors (as percentage of inhibition) on iron and calcium availability from 13 types of green leafy vegetables. They found tannins (17, 18%), oxalates (25, 36%) and phytic acid (16, 1%) to be important inhibitory factors. Dietary

Figure 2. Effect of fruits and processed beverages on available iron from composite meals*



*Note : Data adapted from original paper.

Figure 3. Bioavailable iron from fruit cheese fortified with two types of fortificants*



*Note : Data adapted from original paper.

fiber was not found to be an inhibitory factor and 42-55% of inhibition could not be explained, thereby indicating that there were other factors also which influence inhibition[6]. Dehydrated greens can be incorporated into numerous Indian dishes to increase the nutritional density of the dishes significantly.

Fruits as promoters of iron absorption

Fruits are recognized as promoters of iron absorption. Obviously, this is because they contain ascorbic acid and other organic acids. Consuming a fruit with every meal is a possible strategy to enhance iron absorption. Fruits can also be used in processed products. Figure 2 depicts the effect of either pulp or juice of 4 types of fruits on combination diets of green leafy vegetables, rice and toor dhal. Fruit juice had a better effect than pulp, because of the higher content of acid and lower content of fiber. Surprisingly, similar effects were observed with processed beverages from either natural or synthetic ingredients. This shows the beneficial effect of organic acids on iron absorption[7]. Processed fruit products can also be used as a vehicle for fortification, as the absorption of iron from fruit products is better than from any other product. Salma and Prakash[8] studied the iron bioavailability from iron-fortified fruit cheese (a product made with ripe guava or pineapple, sugar and butter) and the results are presented in Figure 3. As can be seen, nearly 18.5% of iron was bioavailable in fruit cheese fortified with ferrous sulphate, whereas similar figures for ferrous fumarate were 9 and 17% for guava and pineapple cheese, respectively. Added iron fortificants exhibited a high bioavailability because of the fruit matrix[8]. In another study on processed breakfast cereals with added fruits, the iron bioavailability was found to be in the range of 8.5-15.0%, which is much higher than what is seen for traditional cereal products alone, indicating that even in processed products inclusion of fruit enhances iron absorption[9].

Influence of pre-processing techniques on nutrient availability

Milling, sieving and germination are preprocessing techniques which can alter the availability of nutrients by altering the compositional matrix of the food. Table 2 presents decrease / increase in content and availability of minerals in food grains as a result of such processing techniques. The process of refining can decrease the mineral content in the food; this is very important to keep in mind, considering the increased consumption of processed foods based on refined cereals. While the general assumption is that lowering of dietary fiber would enhance the bioavailability of minerals, the fact is that the actual amounts of minerals in the processed products would be much lower. To illustrate this, the results of differential milling of wheat, sieving of finger millet flour and germination of legumes are presented in Table 2[10-12]. As can be seen, differential milling of wheat reduced the zinc and iron content in refined wheat flour, coarse semolina and fine semolina in comparison to whole wheat flour. There was also a reduction in fibre content as expected due to removal of bran. However, bioavailability

Table 2. Effects of pre processing of food grains on bioavailable minerals (per 100g dry weight basis)

Pre-processing techniques		Total minerals		Bioavailable minerals				Dietary Fiber	
		Iron (mg)	Zinc (mg)	Iron (mg)	Percent	Zinc (mg)	Percent	Insoluble (g)	Soluble (g)
Wheat, differential milling[10]									
Whole wheat flour		7.54	1.62	0.25	3.30	0.08	4.94	11.4	0.47
Refined wheat flour		3.24	0.70	0.136	4.20	0.054	7.70	3.00	0.24
Coarse semolina		3.23	0.66	0.115	3.56	0.37	5.61	4.60	0.34
Fine semolina		3.56	0.70	0.110	3.10	0.33	4.71	4.66	0.20
Ragi, milling and sieving[11]									
Whole		6.52	2.50	0.224	3.43	1.57	6.23	20.23	1.55
Sieved		2.39	1.98	0.171	5.23	1.58	7.98	12.15	1.79
Legumes, germination & dehulling[12]									
Constituents		**Iron (mg)**	**Calcium (mg)**	**Iron (mg)**	**Percent**	**Calcium (mg)**	**Percent**	**Insoluble (g)**	**Soluble (g)**
Green gram	Raw	11.14	136	1.21	10.9	21.4	15.7	16.58	3.42
	Germinated	10.15	114	1.86	18.3	28.2	24.7	15.8	4.71
	Germinated & Dehulled	9.05	71	3.23	35.7	28.8	40.5	12.6	2.57
Cowpea	Raw	6.5	87	0.73	11.2	19.7	22.6	25.18	2.19
	Germinated	5.87	75	1.16	19.7	28.7	38.2	25.80	2.30
	Germinated & Dehulled	5.52	54	2.08	37.6	29.6	54.8	27.48	1.34
Lentil	Raw	8.52	77	0.87	10.2	22.6	29.3	15.62	0.85
	Germinated	6.73	63	1.24	18.5	29.3	46.5	15.52	1.48
	Germinated & Dehulled	5.55	50	2.24	40.4	29.8	59.6	11.39	0.34
Chickpea	Raw	4.68	222	0.53	11.3	42.8	19.3	25.91	1.27
	Germinated	3.77	176	0.70	18.6	57.9	32.9	25.56	2.47
	Germinated & Dehulled	2.94	63	1.13	38.6	30.3	48.1	19.69	0.54

Table 3. Effect of processing on mineral bioavailability from fortified cereal products (per 100g dry weight basis)

Product details	Steamed products			Deep fried products		
Rice based products[3]	**Total iron (mg)**	**Bioavailable iron (mg)**	**Percent bioavailable iron**	**Total iron (mg)**	**Bioavailable iron (mg)**	**Percent bioavailable iron**
Control	1.23	0.33	2.69	1.09	0.027	2.50
+ iron, 10mg	11.36	0.74	6.52	11.83	0.614	5.19
+ iron, 20mg	21.90	1.55	7.07	21.85	1.31	5.99
+ RB, 10%	3.39	0.126	3.73	3.72	0.119	3.22
+ RB, 20%	5.70	0.213	3.74	5.82	0.200	3.43
+ iron + RB, 10mg+10%	12.99	0.632	4.54	13.16	0.598	4.54
+ iron + RB, 20mg+ 10%	23.55	1.03	4.38	22.55	1.126	4.99
Finger millet based products[11]	**Total iron (mg)**	**Bioavailable iron (mg)**	**Percent bioavailable iron**	**Total iron (mg)**	**Bioavailable iron (mg)**	**Percent bioavailable iron**
Whole finger millet						
Control	6.36	0.363	5.71	5.91	0.224	3.79
+ 20mg iron	20.73	1.767	8.52	25.64	1.686	6.58
Sieved finger millet						
Control	3.08	0.232	7.5	3.24	0.198	6.11
+ 20mg iron	19.22	1.872	9.7	23.2	1.844	7.95
-	**Total zinc (mg)**	**Bioavailable zinc (mg)**	**Percent bioavailable zinc**	**Total zinc (mg)**	**Bioavailable zinc (mg)**	**Percent bioavailable zinc**
Whole finger millet						
Control	2.05	0.158	7.7	2.15	0.137	6.37
+ 15 mg zinc	13.28	1.184	8.92	14.94	1.162	7.78
Sieved finger millet						
Control	1.89	0.16	8.47	1.87	0.151	8.07
+ 15 mg zinc	13.55	1.536	11.34	14.62	1.29	8.82
RB: rice bran						

Table 4. Effect of adding iron absorption promoters on iron bioavailability from fortified biscuits[13]

Fortified products	Total iron (mg)	Bioavailable iron (mg)	Percent bioavailable iron	Percent increase over control
Control	4.81± 0.8	0.99 ± 0.00	20.58	-
$FeSO_4$	8.75 ± 2.02	1.26 ± 0.07	14.4	27.2
$FeSO_4$+60mg CA	9.18 ±1.6	1.54 ± 0.05	16.78	55.6
$FeSO_4$+80mg CA	8.75 ± 0.0	2.02 ± 0.29	23.08	104
$FeSO_4$+100mg CA	9.62 ± 1.01	1.52 ± 0.30	15.80	53.5
$FeSO_4$+60mg TA	8.75 ±1.42	4.07 ± 0.08	46.51	311
$FeSO_4$+80mg TA	9.18 ±0.87	4.93 ±2.10	53.7	398
$FeSO_4$+100mg TA	9.62 ± 1.01	5.31 ±0.89	55.19	436
NaFe EDTA	10.93 ± 1.67	1.82 ±0.17	16.65	83.8
NaFeEDTA+60mg CA	8.31 ±1.67	1.99 ±0.22	23.95	101
NaFeEDTA+80mg CA	8.75 ±1.42	2.15 ±0.01	24.57	217
NaFeEDTA+100mg CA	10.5 ±2.47	1.62 ±0.38	15.42	63.7
NaFeEDTA+60mg TA	10.5 ±2.47	1.85 ±0.11	17.62	86.9
NaFeEDTA+80mg TA	10.0 ±2.20	2.98± 0.50	29.8	201
NaFeEDTA+100mg TA	9.18 ±0.87	4.34± 0.19	47.28	338.4

CA: Citric acid, TA: Tartaric acid.

studies indicated that the absolute content of available mineral was higher in whole wheat flour although the percentage bioavailability was higher in the refined products. Finger millet flour also has a high fibre content, which can be partially removed by sieving. In sieved flour the bioavailable iron was higher whereas that of zinc remained unchanged; the percentage availability of both minerals was higher in the sieved products. Hence, the presence of fibre does not necessarily reduce mineral bioavailability to a great extent in all conditions. In germinated legumes, the mineral bioavailability increased substantially. While it was already high in whole germinated legumes, dehulling produced a further increase which could be due to reduction in anti-nutritional factors and a change in the matrix due to enzymic action favouring bioavailability.

Nutrient availability from processed products

Selected data on the effects of processing treatments on nutrient bioavailability from fortified rice and finger millet products is given in Table 3. The processing treatments chosen represented two different matrices, i.e. wet matrix in steamed products and dry matrix in deep-fried products. The products were fortified with various levels of iron and zinc. The compositional alteration of the matrix was also effected by addition of rice bran (inclusion of extra fibre to represent processed foods with added fibre) or sieving of flour (to reduce fibre to represent refined flours). The results indicated that the addition of fortificants increases the nutrient content as well as bioavailability of nutrients. Alteration in fibre content had a marginal effect on bioavailability of nutrients, with the type of processing appearing to have an effect. Deep-fried products exhibited lower bioavailability of nutrients in comparison with steamed products[3,11]. The physical properties of the food matrix are subject to change on processing, either hindering or promoting their release from the matrix. One possible explanation for this could be that in a deep-fried product the partially gelatinized matrix is surrounded by a layer of fat and the moisture content is low, thereby reducing the solubility of the nutrient, inhibiting its release from the matrix, and hindering the enzymatic and chemical digestion process, thereby decreasing the availability.

Another study examined the effect of enhancers on iron availability from fortified biscuits[13]. Biscuits were prepared with wheat flour fortified either with ferrous sulphate or ferrous fumarate, and iron absorption enhancers, namely, citric acid or tartaric acid at three concentration levels were added to the product. The addition of enhancers increased the iron bioavailability from fortified products to a remarkable degree. Tartaric acid was more effective than citric acid, increasing the availability almost 3- to 4-fold (Table 4). Hence, the study results indicated that the addition of enhancers to fortified foods can increase iron absorption.

In summary, the nutritional quality of diets can be improved by selecting nutrient-dense ingredients, proper meal combinations, appropriate processing techniques, and the use of enhancers, both in non-fortified and fortified foods. The final form of the food affects the digestibility and absorption of nutrients to a great extent. The bioavailability of essential components could be enhanced by utilizing the food matrix favourably for promoting nutrition security.

The author is from Department of Food Science and Nutrition, University of Mysore, Mysore 570 006, India. [Email: jampr55@hotmail.com]

References

1. Parada J, Aguilera JA. Food microstructure affects the bioavailability of several nutrients. Journal of Food Science, 72, 21-32, 2007.
2. Castenmiller JM, West CE, Linssen JPH, Vanhethof KH, Vorgen AGJ. Food matrix of spinach is a limiting factor in determining the bioavailability of β carotene and to lesser extent of lutein in humans. Journal of Nutrition, 129, 349-355, 1998.
3. Oghbaei M, Prakash J. Effect of compositional alteration of food matrices and processing on availability of selected nutrients and bioactive components in rice products. International Journal of Food Sciences and Nutrition, 62 (3), 250-261, 2011.
4. Oghbaei M, Prakash J. Effects of processing and digestive enzymes on retention, bioaccessibility and antioxidant activity of bioactive components in food mixes based on legumes and green leaves. Food Bioscience, 4, 21-30, 2013.
5. Gupta S, Lakshmi JA, Manjunath MN, Prakash J. Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT, Food Science and Technology, 38 (4), 339-345, 2005.
6. Gupta S, Lakshmi JA, Prakash J. In vitro bioavailability of calcium and iron from selected green leafy vegetables. Journal of Science of Food and Agriculture, 86 (13), 2147-2152, 2006.
7. Lakshmi JA, Gupta S, Prakash J. Effect of processed beverages on bioavailable iron from rice based meals in comparison with fresh fruits. Malaysian Journal of Nutrition, 12 (2), 189-199, 2006.
8. Salma NU, Prakash J. Nutritional composition and sensory quality of iron fortified fruit cheese. Indian Journal of Nutrition and Dietetics, 51 (2), 135-144, 2014.
9. Bhavya SN, Prakash J. Nutritional quality and antioxidant properties of ready-to-eat corn based breakfast cereals. Malaysian Journal of Nutrition, 18 (3), 373-382, 2013.
10. Oghbaei M, Prakash J. Effect of fractional milling of wheat on nutritional quality of milled fractions. Trends in Carbohydrate Research, 5 (1), 53-58, 2013.
11. Oghbaei M, Prakash J. Bioaccessible nutrients and bioactive components from fortified products prepared using finger millet (Eleusine coracana). Journal of Science of Food and Agriculture, 92 (11), 2281-2290, 2012.
12. Ghavidel RA, Prakash J. The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT, Food Science and Technology, 40 (7), 1292-1299, 2007.
13. Thara G, Leelavathi K, Prakash J. Iron bioavailability and sensory quality of iron fortified wheat biscuits. Food and Nutrition Bulletin, 28 (3), 299-306, 2007.

Edited by Dr. Anshu Sharma for the Nutrition Foundation of India, C-13, Qutab Institutional Area, New Delhi - 110016, website : www.nutritionfoundationofindia.res.in
Designed & Printed by Himanshi Enterprises