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Nutritional Strategies for Treating Iron Malnutrition: Implications on Nutrikinetic Approaches

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Abstract

Malnutrition remains a major global issue-affecting people of all ages. Iron deficiency is one of the existing malnutrition deficiencies limiting long-term human development. Food-based interventions play a vital role in the growth, development (physical and cognitive), and socioeconomic status of an individual. Several food-based approaches (viz., fortification and bio fortification of staple foods) have been employed for improving malnutrition in vulnerable populations. In underdeveloped countries, staple foods like rice, wheat, beans, and pearl millets etc. are being actively fortified and bio fortified to enhance the iron content intended to deliver the required nourishment with optimal consumption. However, translational iron levels from functional foods may not be proportional to iron absorbed into systems. Considering the effectiveness of bioavailability, additional emphasis is required in establishing the same for the functional foods which could serve as an effective alternative to existing methods in mitigating iron malnutrition. This review emphasizes the importance of iron, iron physio-pathological, and the current status of food strategies in dealing with iron malnutrition.

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Introduction

Iron is recognized as an essential micronutrient for almost all living organisms since ancient times. Iron as a micronutrient is considered to be a double-edged sword which, in limits help in oxygen transport, DNA synthesis, cellular regeneration, and respiration. But, is toxic in overload states causing oxidative stress and cellular damage.[1] 70% of iron reserves in humans are found in blood haemoglobin and muscle myoglobin. Since the last decade, nutritional disorders associated with the dearth of iron in humans were reported affecting all age groups.

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Thus iron is considered to be the most common form of micronutrient malnutrition (MM) globally.[2,3] Iron deficiencies are the most common MM considered to be a serious public issue affecting over 1.2 billion people globally, especially in pregnant women and children of low- and middle-income countries (Figure1).[4,5,6,7] World Health Organization (WHO) estimates that two billion people are anaemic and attribute approximately 50% of all anaemics to Iron deficiency & Iron deficiency anaemia (ID & IDA).[8] Mounting evidence suggests that the prevalence of iron deficiencies are characterized based on age groups (pediatrics, geriatrics), and gender (mostly women) (Figure 1).[9,10,11,12,13,14] Iron deficiencies not only cause anemia but also leads to premature births, low birth weight babies, and affects overall physical and mental health.[15,16,17] It is well documented that ID could be controlled by food along with a balanced diet in healthy humans. Poor health, increased vulnerability to various diseases, and a significant reduction in annual Gross Domestic Product (GDP), up to 11% in Asia and Africa, are all consequences of inadequate consumption of a healthy diet.[18]

The recommended daily intake of iron varies among different age groups including infants (11 mg), premenopausal women (18 mg), pregnancy (27 mg), and adult men (8 mg).[19] The present review emphasizes a detailed note on the importance of iron, iron physiology and pathophysiology, the current nutrikinetic status of iron in different food interventions, and their future in dealing with iron malnutrition.

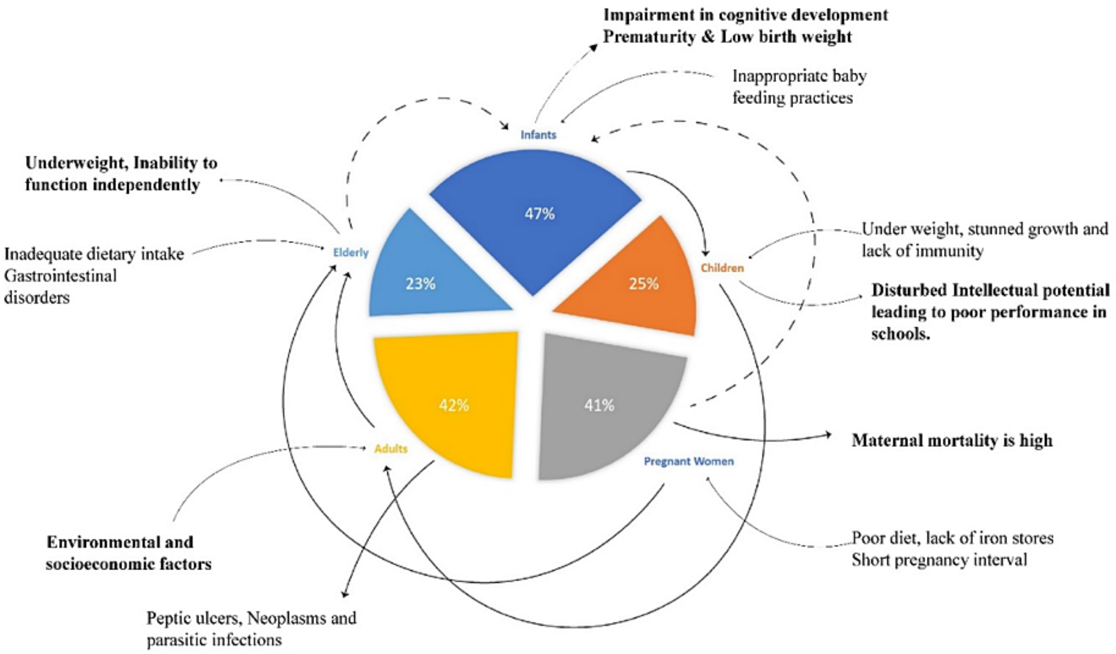

Fig. 1: Global distribution of Iron Deficiency among various age groups, their causative effects, and their associated disorders.

Methodology

In the present systematic review, we have collected published articles from databases including a.) Web of Science b.) Elsevier c.) Scopus. Search engines like Google scholar, PubMed, INFLIBNET were used. Data was collected from research and review articles published between 1991 and 2021. Search keywords includes food fortification, bio fortification, iron fortification, iron nutrikinetics, iron bioavailability, and iron bio accessibility etc. The articles were chosen based on the relevance to the current review, with topics including iron malnutrition, iron deficiency anemia, WHO food interventions, socioeconomic status etc.

Physiological and Pathological Role of Iron

Iron from the diet is either sequestered as heme or nonheme forms. Non-heme forms are poorly absorbed whereas heme forms are most absorbed from basal membranes. In the intestine, nonheme iron, especially in ferrous state (Fe^{2+}), gets absorbed into blood circulation via divalent metal transporter 1 (DMT-1). As the dietary iron is in ferric state (Fe^{3+}), it gets transported across enterocyte basolateral membrane via FPN1 thereby getting reduced from Fe^{3+} to Fe^{2+} by the enzyme copper-dependent iron oxidase hephaestin (Figure 2).[20] The absorbed iron bounds to plasma transferrin for further distribution to the utilization sites. Most of the transported iron is utilized by newly formed red blood cells in the bone marrow. Senescent red blood cells get phagocytosed by macrophages, causing catabolism of hemoglobin for releasing heme form which is transported to bone marrow.[21] Hepcidin, a liver-derived peptide, regulates body iron demand for intake and distribution to enterocytes, macrophages, and other body cells. Free iron (Fe^{3+}) gets sequestered with ferritin, an iron storage protein, then mixing with peptide hemosiderin, for storage and reuse in physiological functions. Defective transporters (i.e., DMT-1, FPN1, transferrin), deficiency of oxidizing enzymes, and lack of iron storage proteins are the key pathological factors that cause iron deficiencies.[22]

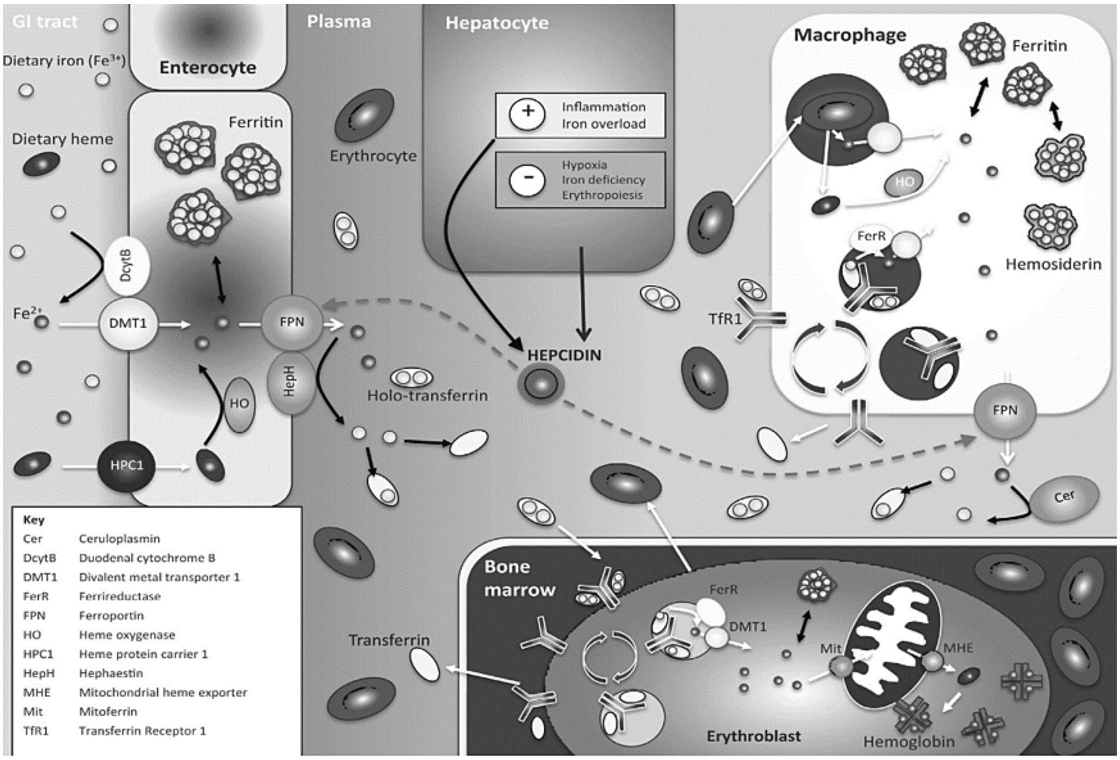

Fig. 2: Iron Physiology. Reprinted from Copyright (2016) with permission from Elsevier Ltd.[23]

Strategies for Enriching Iron Levels in Tackling ID and IDA

Heme and non-heme iron are the two forms available in food sources with different absorption rates in humans. Meat, fish, and seafood are the best sources of heme iron with 15-35% of the absorption rate. Seeds, grains, nuts, and green leafy vegetables majorly contribute to the non-heme iron but have a less absorption rate of 2-20%.[24,25,26] To combat ID and IDA, governing bodies of various countries have taken different initiatives *viz.*, iron supplements in the form of tablets, capsules, and syrups, dietary diversification including iron-rich foods. Also, initiatives for enhancement of iron absorption, fortified iron-rich foods, bio fortification of staple crops are encouraged.[27]

Iron supplementation with ferrous ion salts is considered a first-line treatment and is a provision for large doses of micronutrients, usually in the form of tablets, capsules, or syrups. It is considered the fastest means to control the micronutrient deficient population groups due to its low cost, high bioavailability, and effectiveness. The maternal and child nutrition report in the Lancet series documented that the iron supplementation trial reduced 67% of IDA in pregnant women.[28] In another study, non-pregnant women of reproductive age showed a 27% reduced risk of anaemia by intermittent iron supplementation.[29] Despite different success stories, a substantial proportion of the population consuming ferrous ion salts suffer from gastrointestinal side effects like nausea, epigastric pain, and constipation due to the higher iron doses administered leading to poor compliance.[30,31] Adding to this, routine iron supplementation could lead to potential adverse effects on children in malaria-endemic areas followed by iron overload.[32]

Dietary diversification is also a potent approach that reinforces diet quality by targeting multiple nutrients and emphasizing food groups and food synergy. In rural India, an intervention study was carried out and found that green leafy vegetables consumption more than twice weekly improved the iron content significantly from 44.7% to 60.6%, as did the consumption of seasonal fruits.[33] Lack of evidence-based and measurable endpoints, issues of affordability, poor access to prenatal care, lack of public awareness, poor literacy and insufficient counselling made this approach difficult during implementation.[34,35]

As per WHO, fortification of iron is referred to the practice of elevating the iron content intentionally in food for improving the nutritional quality for public health.[34] Iron fortification technique has gained immense importance in high-income countries for its proven, safe and cost-effective strategy improving human diets and preventing ID and IDA. Mass fortification, open market (commercial) fortification, targeted (high-risk groups) fortification, household fortification, and community fortification methods are five WHO recommended types of iron fortification.[36] The solubility factor of the iron fortificants play an important role in the bioavailability of iron in humans. Iron often reacts with the food components causing organoleptic changes and leading to the oxidation of fats. Therefore, less soluble compounds which are recognized by the generally recognized as safe (GRAS) are often chosen for the fortification. Reduced iron, ferric phosphate, ferric pyrophosphate, etc., are the compounds that are generally recognized by the GRAS for iron fortification purposes which are closer to the physiological requirement.[37] Food staples, condiments, and commercially processed items are the three categories where micronutrients are administrated through food fortification, aimed by improving the nutritional status.[38] Iron fortification criteria for wheat and maize flours have now been widely accepted due to its wide consumption by larger populations.[39]

In recent years, bio fortification of staple crops seeks to be a rural-based potent, feasible and cost-effective strategy to combat ID and IDA.[40,41] Iron bio fortification is the process of increasing the iron density of food crops through conventional plant breeding and/or improved agronomic practices/ modern biotechnology with no loss in consumer or farmer-preferred characteristics. Bio fortification aims to increase the nutrient content from the plant germination phase rather than during processing of food crops. These cultivars have a positive influence over agro-economical, nutritional and functional health outcomes.[42] Agronomic, conventional and transgenic are the common approaches to biofortification.[43,44] Ferritin, a protein that is nontoxic and present in plants had been expressed through genetic modifications to combat iron deficiencies.[45] For instance, overexpression of the OsIRT1 gene (a divalent metal transporter) in rice increased iron concentration levels by 1.1 folds in rice grains.[46] Similarly in cassava tubers, combined overexpression of IRT1 and Fer1 gene increased iron by 5.5 folds.[47] The countries where poor staple crop dominate the diets, they are being successfully bio fortified with iron for contending ID and IDA.[48,49] Considering the benefits and limitations, bio fortification has many advantages over fortification in many aspects. i.e., cost-effectiveness, sustainability, major impact on remote and rural areas, providing long-term means of delivering micronutrients followed by less toxic effects.

Nutrikinetic Profiling of Iron-Enriched Food Varieties: *In Vitro*, Pre-Clinical and Clinical Evidences

Nutrition plays a crucial role in the healthy growth, development (physical and cognitive), and socioeconomic status of humans. Though many studies have witnessed the effects of specific bioactive nutrients relative to specific complications, a holistic food-based approach containing essential nutrients remains to be a key strategy to alleviate malnutrition.[50]

Functional foods such as whole, fortified, bio fortified, enriched, or enhanced foods provide health benefits when consumed at efficacious levels on a regular basis.[51] Poor absorption, food-food interactions, and the poor bioavailability of iron upon consumption remain the major failure of functional foods.[52] Nutrikinetics is considered to be a new approach that mainly focuses on human nutritional ADME, involving identification, time-course analysis, and parameterization of food compounds in human bio fluids. Nutrikinetic assessment for these functional foods help in overcoming the limitations and also lowers the risk of malnutrition.[53] Enhancing the nutrition profile of staple foods through fortification and bio fortification are found to be a promising approach in managing the lacuna of micronutrient deficiencies. In fortification, synthetic forms of iron such as ferrous sulphate, ferrous pyrophosphate, sodium feredetate, ferric sodium ethylenediaminetetra acetate (NaFeEDTA), etc. are supplemented and mixed with staples under controlled supervision in the food industry whereas, in bio fortification, a purposeful increase of these micronutrients through conventional breeding or biotechnological methods are employed. Thus, there may be variation in the release profile of these micronutrients since fortification is a process of physical mixture of iron with staples and bio fortification is a process of enrichment of micronutrients during germination and growth phase of the cultivars. This variation may have an impact that can cause the difference in the release kinetics leading to variation in the bioavailability and bio accessibility of micronutrients. In high-phytate diets, absorption of iron from fortified NaFeEDTA is 2-3 times greater than the absorption from ferrous sulfate.[54]

To date, many bio accessibility and bioavailability studies are been carried out independently utilizing different fortified and bio fortified foods with different methods *viz.*, solubility/digestibility, cell line studies (Caco2 cell lines and combination of Caco2 along with HepG2 cell models), haemoglobin repletion methods, and *in vivo* studies (Wistar rats, domestic chicken, rabbits).[55,56,57,58]

A recent bioavailability study on iron-fortified chickpea was carried out and concluded that iron bioavailability in cooked chickpeas (soup and desi chapatti) was increased (5.8-10.5, 15.3-25.0, and 4.8-9.0 ng ferritin/ mg protein) than the raw products and non-fortified product.[59] The bioavailability and bio accessibility of iron bio fortified cowpea study were carried and compared with cooking and raw (germinated) conditions which concluded that the iron absorption was higher in germinated (raw) when compared to cooked conditions through *in vitro* iron bio accessibility studies.[60] Therefore, it is of prime importance to establish a comparative data regarding the bio accessibility and bioavailability of these micronutrients available as fortified foods & bio-fortified cultivars to reach the anticipated target effect.[61,62]

In Vitro Evidences

Due to their significance and broad applicability, *in vitro* models of human tissues are gaining popularity. A combination of cell culture and *in vitro* digestive models provides an alternative to human and animal research which is sometimes difficult to undertake due to ethical considerations. Many *in-vitro* studies have been performed to understand the iron bioavailability from various fortified and bio fortified food varieties such as soybean, rice, wheat, chickpeas, etc. Fumiyuki Goto *et al.,*[63] and colleagues had expressed soybean ferritin in rice seeds through Agrobacterium mediated transformations which leads to an increase by three folds of iron content in the grain which was furtherly conformed with southern blot analysis. Diane M. Della Valle *et al.,*[64] have investigated 23 bio fortified lentil genotypes from different localities for iron concentration, and bioavailability using *in vitro* cell lines and established a baseline for future improvements in iron bioavailability. In addition, an *in-vitro* study was carried out on sixty-seven marketed beans and 150 bio fortified varieties in East Africa. The author concluded that the approach of high iron bean bio fortification approach couldn't meet the malnutrition requirements and many bio fortified varieties remained no different from normal variety.[65] Tammanna A Jaan and colleagues[59] studied and examined chickpea potentiality by fortifying iron with ($FeSO_4.7H_20$) and NaFeEDTA through spraying and drying methods. Under various cooking conditions, cooked split desi seeds (soup), desi chapatti, and Kabuli chapatti were made with iron-fortified chickpea, their respective iron bioavailability was increased by .5.8-10.5, 15.3-25.0, and 4.8-9.0. ng ferritin/mg protein. In another study, a simple *in vitro* technique was developed to improve iron and zinc content in chickpeas i.e., by soaking chickpeas in fortified water. This method was reported to improve micronutrient mineral content, and higher bio accessibility even under cooking conditions.[66] Rateesh and colleagues[67] investigated the inhibitory factor variations in 13 bio fortified pearl millets and found that the inhibitors are majorly affecting the bio accessibility of micronutrients. Further, the grains when subjected to soaking or fermentation before consumption might lead to improvement in the bio accessibility of the micronutrients. Selection of food variety for fortification plays a crucial role in reducing malnutrition. For instance, in a study, biscuits which are fortified with iron showed good bioavailability but upon storage, damaged the shelf life and lead to lipid oxidation.[68] Apart from traditional methods, novel fortification techniques like cold plasma treatment, iron binding peptides, iron nanoparticles, and iron polymeric liposome encapsulation have also been successfully employed to improve bio accessibility in different food varieties.[69,70,71,72]

Pre-Clinical Evidences

Preclinical studies are considered to be an appropriate model for evaluating the bioavailability and bio accessibility of enriched foods using animal models. In a study, Cintia Tamez Sunt Ana *et al.,*[60] demonstrated that pro-vitamin A had no discernible impact on the bioavailability of iron in the bio fortified cowpea and cassava mixture according to *in vivo* methods, which also revealed a 19.5 percent increase in iron content in the bio fortified variety. Few research studies were performed to assess the toxicity of excessive and prolonged intake of fortified dietary iron in male wister rats. The results had shown significant evidence for toxicity, and oxidative stress which may cause colon-related problems in humans. Studies also proved that excessive storage of iron (Iron overload) would lead to cardiomyopathy and is considered a major factor of morbidity and mortality among humans.[73,74]

Clinical Evidences

Despite of expensiveness and ethical considerations, human trials are considered to be more accurate and precise for evaluating iron from enriched varieties. Sumitra Muthayya *et al.,*[75] had carried out randomized efficacy trials on iron-deficient school-going children in India with fortified wheat and found that in the treatment group, the prevalence of ID and ID anaemia decreased significantly from 62 to 21 percent and 18 to 9 percent, respectively. They also reported improving results in reducing iron deficiency and body iron stores. Another study was performed to find the role of citric acid in iron-fortified fish sauce on 10 adult women and reported that iron absorption was higher in the presence of citric acid with 14.1% when compared to 12.0% in its absence.[76]

Pauline and their colleagues[77] conducted a randomized study on 516 iron-deficient children by dividing them into three groups. The first group received high dose of NaFeEDTA fortified maize flour (56 mg/kg), the second group received a low dose (28 mg/kg) and the third group received elemental iron (56 mg/kg). According to the study's findings, consuming high doses of NaFeEDTA helped children's iron status indicators by lowering ID and IDA.

Randomized efficacy trials have also been carried out in different age groups with bio fortified cultivars like rice, beans, and pearl millets. In these studies, it was evidenced to be a promising approach against iron deficiencies.[78,79,80,81] For example, in a study, the authors investigated to what extent the iron is bioavailable in both post-harvest fortified and bio fortified pearl millets and found that post-harvest iron-fortified millet meals (10.4 percent) had higher fractional iron absorption when compared to regular-iron bio fortified millet meals.[82] Similar studies on pearl millets was carried out in the same year on young children and found enhancing concentrations of bioavailable iron when consumed as primary staple food.[81] Petry *et.al.,*[83] conducted an iron absorption study in cooked composite meals with bio fortified beans which resulted 54 percent higher concentration of iron than in the same meals with control beans. To compare the iron absorption from meals containing bio fortified beans (8.8 mg Fe/100 g) and control beans (5.4 mg Fe/100 g), a crossover design study involving 22 Rwandese women was conducted. This study also demonstrated high levels of Fe absorption. Iron absorption inhibitors such as phytic acid constrained the bioavailability of the iron in bio fortified beans.

Few research studies suggested that a combinational approach i.e., combining iron with vitamin A could potentiate the bioavailability in both fortified and bio fortified food varieties. For instance, an *in vivo* study described the effect of combinations of bio fortified beans and rice with high carotenoid content. This showed an increase in iron bioavailability, favourably influenced by increased plasma antioxidant capacity and protein genes involved in iron metabolism.[84] Similar work was carried out by one of the authors in fortified pearl millet weaning foods bio fortified with iron along with vitamin A and also investigated the role of vitamin A in iron absorption. However, the results obtained were highly promising and maintained iron homeostasis and hepatic iron stores in animals with low levels of iron as well as significantly increased the digestive and metabolic utilisation of iron.[85] Laura and their colleagues[86] carried out a randomized intervention study on iron bio fortified beans with 150 low iron status women and concluded that consumption of iron-bio fortified beans improved cognitive performance in young adult women.

Table 1: Few Examples on Food Strategies that were Employed for Iron Malnutrition.

S. No.	Type of food vehicle	Food intervention	Outcome	Study Design	Reference
1.	Beans	Biofortification	The bio fortified beans had improved cognitive performance in young adult women	Clinical	86
2.	Chick peas	Fortification	Fe bioavailability > by 5.8–10.5, 15.3–25.0, and 4.8–9.0 ng ferritin/mg protein	*In vitro* approach	59
3.	Wheat Biscuits	Fortification	Food fortification with iron and EDTA - decreased blood lead concentrations. NaFeEDTA should be the iron fortifier of choice.	Clinical	93
4.	Wheat	Fortification	Decreased anaemia from 62 to 21 percent and 18 to 9 percent in children	Clinical	75
5.	Peral Millets	Fortification & Biofortification	The fractional iron absorption of post-harvest iron-fortified millet meals was higher (10.4 percent)than that of regular and iron- bio fortified millet meals.	Clinical	82
6.	Polished Rice	Fortification	The bio fortified rice had more bioavailable iron than the unfortified rice.	*In vitro*	94
7.	Beans	Biofortification	Fe-bio fortified beans had a lower bioavailability than regular beans.	*In vivo* approach	95
8.	Pearl Millet	Fortification	The liver's iron stores returned to normal levels as iron bioavailability increased.	*In vivo* approach	85

Limitations of Fortified and Bio Fortified Food Interventions

Despite their advantages, still there exist some challenges for fortified and bio fortified food varieties such as iron overload, labour-intensive effort, improper selection of food vehicles, stability issues, and involvement of expensive technologies, etc.[48,87] Digestibility, stability, bio accessibility, and bioavailability of the functional foods are highly influenced by cooking procedures, food matrix, and the presence of micronutrient enhancers and inhibitors.[88]

The nutritional and anti-nutritional factors considerably improve or inhibit iron absorption when ingested in different forms. Phenolic compounds and phytates are some of the anti nutritional compounds present in plant-based foods that hinder iron absorption in humans. Animal proteins such as milk, soybean, and albumin also reduce iron absorption. Black tea and coffee consumption has also been shown to significantly reduce the amount of iron that is absorbed from composite meals, with coffee having about half the inhibitory effect of tea.[89] On the other hand, research revealed that vitamin C significantly promotes the absorption and bioavailability of iron and is shown to be more effective at increasing iron status. In vegetarians, the effect of vitamin C, which is itself an antioxidant, acts significantly to counteract the effect of phytic acid.[90] Additionally, consumption of meat, fish, and poultry increases the absorption of nonheme iron.[24] Moreover, further research focus on this optimal intake, reducing levels of anti nutrients in functional foods could be the smart approach for improving iron absorption and bioavailability.[91,92]

Conclusion

Overall, the above-mentioned strategies provide us with in-depth knowledge on the application of functional foods for improving iron levels. A significant barrier to food fortification with stable and bioavailable nutrients without compromising organoleptic qualities is the current lack of accessible, low-cost technology. Lastly, fortification failed to bridge the low-income and suburban areas. Similarly, the major drawback in bio fortification is the kinetic correlation between consumption and bioavailability among affected population. Further research should focus on developing comparative nutrikinetic aspects in the same or diverse functional food varieties (fortified vs fortified; bio fortified vs bio fortified, bio fortified vs fortified) which could provide new insights into assessing and evaluating health outcomes, cost-effectiveness, bioavailability, bio accessibility, and toxicity.

Author Contributions

Conceptualization - Bhadram Kalyan Chekraverthy, Krishnaveni Nagappan, Literature search - Bhadram Kalyan Chekraverthy, Ammu V. V. V. Ravi Kiran, Harshita Arun Pardhe; Data analysis - Bhadram Kalyan Chekraverthy, Ammu V. V. V. Ravi Kiran; Drafting Manuscript - Bhadram Kalyan Chekraverthy, Ammu V. V. V. Ravi Kiran, Harshita Arun Pardhe; Supervision & Proof-reading - Krishnaveni Nagappan

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Conflict of Interest

The authors state that there is no conflict of interest.

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