

## Advanced Insights into The Role of Food Processing, Fortification and Antinutritional Factors on Iron Absorption

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### ABSTRACT

Iron deficiency remains a widespread global health concern, particularly in populations consuming predominantly plant-based diets, where iron bioavailability is often compromised due to the presence of antinutritional factors. This systematic review evaluates the effectiveness of various strategies to enhance iron bioavailability, focusing on food processing methods, iron fortification, and the impact of dietary inhibitors. Using the PRISMA methodology, relevant studies were systematically identified, screened, and analysed. The review examines the influence of different food processing techniques, including heat processing, germination/malting, milling, soaking, and fermentation, in reducing the inhibitory effects of phytates, polyphenols, and tannins. These methods have been shown to modify food matrices, improve iron solubility, and enhance its absorption. The role of iron fortification is also examined, with a focus on selecting appropriate iron compounds and incorporating complementary dietary strategies that facilitate better iron uptake. Additionally, the review investigates the impact of antinutritional factors dietary fiber, phytates, polyphenols, tannins, and lectins on iron absorption and discusses strategies to mitigate their effects. The findings from the analysed studies highlight the importance of optimizing food processing techniques and fortification strategies to improve dietary iron availability. The review concludes by summarizing the results of three key research questions, offering insights that can guide dietary recommendations and nutritional strategies for addressing iron deficiency.

**Keywords:** Iron bioavailability, food processing, fortification, anti-nutritional factors, PRISMA.

### 1. INTRODUCTION

The essentiality of iron has been known since ancient times and its beneficial effect on blood formation was recognized in the 17<sup>th</sup> century (Henry & Miller, 1995). Iron has many special functions in the body. More than 65 percent of the body's iron is in the blood in the form of haemoglobin, which is a protein in red blood cells that transports oxygen to tissues in the body (Abera Teka, 2019). There are two forms of iron, heme and non-heme, found in foods. Heme iron is found only in animal foodstuffs such as meat, fish, and poultry products, whereas non-heme iron is present in fruits, vegetables, dried beans, and grain products. Heme iron is absorbed with better proficiency by the intestine as compared to non-heme iron. The average daily dietary iron intake is 10 to 15 mg in humans, however, only 1 to 2 mg iron gets absorbed through the intestinal system (Piskin et al., 2022). As of this moment, it is sufficiently recognized that iron bioavailability from ongoing Indian diets is low due to elevated phytate level and low ascorbic acid/iron ratios. These variables are instrumental in defining iron bioavailability and the recommended dietary allowances (RDAs). Significant distinctions can be observed in the composition of the iron recommended dietary allowances among the physiological groups, which need to be validated (Nair & Iyengar, 2009). When the food is ingested, the nutrients are released from the food matrix and converted into absorbable units, which are then transported into the bloodstream and delivered to their target tissues. The nutrient fraction which is released from the food matrix and available for absorption is referred to as bioaccessible (which can be, for example, determined *in vitro*),

whereas the nutrient fraction which is absorbed and available for utilization in physiologic functions and storage is referred to as bioavailable (examined by *in vivo* experiments or cell cultures) (Rousseau et al., 2020). The importance of iron in human nutrition and the prevalence of iron deficiency among human populations have encouraged research on the bioavailability of iron (Henry & Miller, 1995).

Household processes such as dehulling, mechanical processing, soaking, germination, and fermentation alone or with thermal processing could influence the bioaccessibility of minerals (Cilla et al., 2019). Processing (including formulation) makes food better, reliable, delicious and extra shelf stable. While the advantages are abundant, processing can also be unfavourable, altering the nutritional attribute of foodstuffs. The time and temperature of processing, product composition and storage are all factors that substantially impact the vitamin status of our foods (Reddy & Love, 1999). Several elements in food can increase or inhibit iron absorption from the diet. Elements such as meat proteins and organic acids enhance iron absorption, while phytate, calcium and polyphenols decrease iron absorption. Iron levels in the body are tightly regulated since both iron overload and iron deficiency can exert harmful effects on human health (Perera et al., 2023). Dietary iron absorption is mostly implemented through enterocyte cells on the duodenum and upper jejunum of the small intestine. Because humans do not have an active iron excretion system, intestinal iron absorption is critical for maintaining iron balance in the body (Piskin et al., 2022).

The World Health Organization stated that iron deficiency anemia is one of the most prevalent nutrient deficiencies in the world. Various factors may affect its absorption like minimal dietary intake of iron, deprived iron absorption, or extreme blood loss. Moreover, polyphenolic compounds widely found in coffee and tea such as chlorogenic acids, monomeric flavonoids, and polyphenol polymerization products also strongly inhibit dietary non-heme iron absorption (Abera Teka, 2019). Individuals are at increased risk of iron deficiency due to factors such as age, pregnancy, menstruation and various diseases. Different solutions of iron deficiency have been applied at individual and community levels. Iron supplements and intravenous iron can be used to treat individuals with iron deficiency, while various types of iron-fortified foods and biofortified crops can also be employed for larger communities (Perera et al., 2023). Various studies have been carried out for years to improve iron bioavailability and combat iron deficiency. In addition to traditional methods, innovative techniques are being developed day by day to enhance iron bioavailability (Piskin et al., 2022). This review paper summarizes the effects of different processing techniques on iron bioavailability. Further, this review also investigates the effect of food fortification and antinutritional factors on the iron bioavailability.

2. EFFECT OF FOOD PROCESSING ON IRON BIOAVAILABILITY

Food processing can enhance food safety and stability, it can initiate desired process-induced flavours and can result in palatability enhancement. Although there are many advantages, but processing can also be detrimental, for example, by initiating unwanted changes such as loss of colour, flavour, texture and smell. In addition, processing can affect the nutritional quality of foods. The nutritional quality of minerals in foods be determined by their quantity as well as their bioaccessibility and bioavailability (Rousseau et al., 2020). Minerals, unlike vitamins, are not demolished by heat, light, oxidizing agents or pH. However, minerals can either be separated from foods during processing (leaching, physical separation) or can be included from the devices used to process foods. The bioavailability of minerals mainly iron (Table 1) is mostly altered by the processes of milling, soaking, cooking, germination, fermentation, and heat processing. All the above processes influence mineral bioavailability either directly by affecting their solubility or indirectly by destroying the inhibitory effect of phytic acid or tannins (Reddy & Love, 1999).

Table 1. Overview of Key Findings and Effect of food Processing Techniques on Iron Bioavailability.

Author/Year	Processing Methods	Objectives	Key Findings	Limitations
Gibson et al. (2006); Moyo (2024)	Heat Processing	To assess the effect of heat processing on mineral bioavailability	Heat processing reduces inhibitors to increase iron bioavailability.	Excessive heat may destroy heat-sensitive vitamins and degrade food texture
Samtiya et al. (2020)	Germination/Malting	To ascertain how germination and malting affect the absorption of iron	Iron bioavailability in grains and legumes is greatly increased by germination.	Long germination periods may lead to nutrient losses and microbial contamination

<b>Moyo (2024)</b>	Milling	To assess the impact of milling on iron bioavailability	Milling enhances iron absorption by removing inhibitory compounds	Can also eliminate both antinutrients and healthy nutrients.
<b>Popova &amp; Mihaylova (2019); Rousseau et al. (2020)</b>	Soaking	To study the effect of soaking on mineral retention	By lowering inhibitors, soaking increases iron bioavailability.	May lead to loss of water-soluble minerals
<b>Popova &amp; Mihaylova (2019); Samtiya et al. (2020)</b>	Fermentation	To analyze the role of fermentation in enhancing iron bioavailability	Fermentation breaks down phytic acid, increasing iron bioavailability.	Requires regulated settings to avoid microbial deterioration.

This table presents various food processing methods and their impact on iron bioavailability, highlighting key findings and associated limitations.

### 2.1. Heat Processing

Heat processing can affect bioavailability by changing mineral solubility and by destroying food constituents that either increase or decrease availability (Reddy & Love, 1999). The heat treatments, such as cooking, pasteurization, sterilization, microwave heating and roasting, are more extensively used. These techniques all differ in temperature-time profiles obtained during the process, as well as by the use of dry or wet heating technologies (Rousseau et al., 2020). Cooking and baking can destroy ascorbic acid and its effect on iron availability. Cooking is one of the easy and commonly used thermal treatment for inactivation of anti-nutrients (Devi et al., 2019). Cooking might improve mineral bioavailability by increasing solubility due to cell wall disruption, protein denaturation and release of organic acids. For example, iron bioavailability increased by at least 200% when vegetables such as broccoli, kale and cabbage were cooked (Reddy & Love, 1999). Cooking whole grains, beans and vegetables can reduce certain antinutrients such as phytic acid, tannins, and oxalic acid (Popova & Mihaylova, 2019). Most of the foods showed health benefits when consumed after autoclaving. For example, boiling of food grains reduced anti-nutrients content, which improved their nutritional value. Most of the previous studies concluded that autoclaving is the best method to reduce levels of several anti-nutritional compounds when compared to other processing methods (Samtiya et al., 2020).

Along with this one study reported that autoclaving decreased phytic acid in dry bean, chick pea and black gram, cowpea and black bean (Devi et al., 2019). Another study have shown that heat processing has differing effects on different forms of iron. Iron absorption in humans from chocolate milk powder fortified with ferrous fumarate was two-fold higher than for ferrous sulphate when the ferrous fumarate was added to the milk before it was dried (under vacuum for 3 h at 95°C). When ferrous fumarate was added to the milk after drying, absorption of the iron compound was only 1.2-fold higher than that of ferrous sulphate. In contrast, iron absorption from ferric pyrophosphate was reduced by 72% when it was added to the milk before processing (Reddy & Love, 1999).

### 2.2. Germination and malting

Germination commonly changes the nutritional level, biochemical property and physical features of the foods. Germinated cereals showed enhanced activity of phytase-degrading enzyme while in non-germinated cereals the endogenous activity of phytase enzyme was observed in diminished amounts (Samtiya et al., 2020). The loss of phenolic compounds during germination could be due to enzymatic activity (polyphenol oxidase), conversion into other phenolics, leaching, and complex formation with proteins (Rousseau et al., 2020). Malting leads to an increase in phytase activity in certain cereals (e.g. maize, millet and sorghum) in most legumes and in oil seeds through de novo synthesis and/or activation of intrinsic phytase (Gibson et al., 2006). Phytase activity during germination is needed to mobilize phosphorus from phytic acid, as it is required for the growth of the new seed (Rousseau et al., 2020). Reduction of anti-nutrients like tannin and phytic acid in germinated cereals increase the bioavailability of several minerals, which led to increased nutritional value of the food products (Samtiya et al., 2020).

One study reported that malting of millet reduces 23.9 % phytic acid after 72 h and 45.3 % after 96 h of germination. The

greatest reduction of phytic acid phosphorus has been found in rye while smallest decrease was observed in maize (Devi et al., 2019). Malting of weaning foods (wheat, barley and green gram) increased iron bioavailability by 16-32%. Iron and zinc absorption in humans were greatly improved by reducing phytate levels by soaking and malting breakfast cereal made from oats (Reddy & Love, 1999). Another study reported that germination of food grains had contrasting effects on the bioaccessibility of zinc and iron. Zinc bioaccessibility of chickpea was not affected while it was significantly decreased in the case of finger millet (up to 38%) and green gram (up to 44%). Iron bioaccessibility increased by 62% in green gram, by 39% in chickpea and by 20% in finger millet after germination. It was observed that in general the impact of germination on zinc bioaccessibility is much lower (or even negative) compared to the one observed for iron (Rousseau et al., 2020).

### 2.3. Milling

The process of dehulling and splitting of cotyledons of pulses into two halves is known as milling (Devi et al., 2019). The milling technique removes anti-nutrients (e.g. phytic acid, lectins, tannins), which are present in the bran of grains, but this technique has a main disadvantage that it also removes important minerals (Samtiya et al., 2020). In process of milling, the antinutrients which are present in seed coat can easily be removed due to mechanical force applied during milling. Tannins can be removed easily by dehulling or milling as it is primarily located at seed coat of dry seeds resulting in improvement of nutritional quality of protein (Devi et al., 2019). One research found that iron absorption was three-fold higher at the lower range than at the higher range of phytic phosphorus concentrations. Pearling of sorghum also increases iron absorption due to the reduction in polyphenol and phytate content. With this dehulling of soy beans reduced iron absorption since the iron in soy hulls was similar to that of  $\text{FeSO}_4$ , a highly available form (Reddy & Love, 1999).

Milling, a processing technique generally employed on raw cereal grains, has the intent to crush the grain into flour, whether merged with a (complete) partition of bran and germ from the endosperm. The effect of separation of bran and germ from the endosperm during milling can also influence the mineral concentration and mineral bioavailability (Rousseau et al., 2020). One study reported that transformation of whole wheat to white flour consequences in 16-86% loss of iron, zinc, copper, magnesium and selenium. While losses of minerals are significant during milling, mineral bioavailability can enhance due to the decrease in the phytic acid/bran content of the grain. Iron absorption from the bread made with white flour was 23% compared to 3% absorption from the same bread made with bran, suggesting that either the fibre or phytic acid in bran was responsible for the reduction in iron absorption (Reddy & Love, 1999). However, not only minerals are removed since antinutrients like phytic acid, dietary fibre and tannins, are mainly found in the bran and germ fraction (Rousseau et al., 2020).

### 2.4. Soaking

Soaking is an attractive method for removing anti-nutrient content of foods because it also reduces cooking time (Samtiya et al., 2020). Soaking can be seen as one of the easiest physical processes to remove soluble antinutritional factors (Popova & Mihaylova, 2019). In soaking process, an increase in phytate degradation of 4-50% is observed in sorghum and millet, depending on the type of pulse or cereal grain studied (Rousseau et al., 2020). Another study reported that soaking cereals such as pearl millet with endogenous or exogenous phytase enzymes, at optimum conditions, significantly improved the in vitro bioaccessibility of iron and zinc by 2-23% (Rousseau et al., 2020). The soluble antinutrients could be reduced by soaking either in plain water or in salt solution like sodium bicarbonate ( $\text{NaHCO}_3$ ) (Devi et al., 2019). Soaking generally provides essential moist conditions in nuts, grains and other edible seeds, which are required for their germination and associated reductions in level of enzyme inhibitors as well as other anti-nutrients to enhance digestibility and nutritional value. Soaking is also commonly required for fermentation, which can also be used to reduce the level of various anti-nutrients in foods (Samtiya et al., 2020). A major disadvantage of the soaking is that for a process that takes 96 hours, water soluble protein may loss in the discarded water, reducing the protein content by up to 26.3% (Devi et al., 2019).

Iron and zinc absorption in humans were greatly improved by reducing phytate levels by soaking and malting breakfast cereal made from oats (Reddy & Love, 1999). Another study showed that soaking reduced phytate content significantly at 45 °C and 65 °C. Soaking of grains and beans was found much effective to enhance the minerals concentration and protein availability, accompanied by reductions in phytic acid level (Samtiya et al., 2020). Soaking has the potential to eliminate some antinutrients, which can be destroyed with the discarded soaking medium. However, the amount of eliminated antinutrients varies on the antinutrient type, pH and length and circumstances of soaking. Although it has been observed that soaking has the advantage of decreasing phytic acid and polyphenol levels of for example brown rice and pulses simultaneously, it can also cause undesirable losses of minerals such as iron and zinc (Rousseau et al., 2020). Another study reported that phytic acid concentration in chickpea was decreased by 47.45 to 55.71% when the soaking time was increased from 2 to 12h (Samtiya et al., 2020).

### 2.5. Fermentation

Fermentation improves the bioavailability of minerals not only by reducing the phytate content but also by producing lactic acid which improves mineral solubility (Reddy & Love, 1999). Some of the microorganisms related with fermented foods, be able to produce enzymes (e.g. phytase and polyphenol oxidase) which can destroy antinutrients such as phytic acid and

polyphenols. Amalgamation of these organisms into starter cultures may, therefore, upgrade mineral bioaccessibility and bioavailability. Besides degrading phytic acid and polyphenol content, fermentation could also improve mineral bioavailability by virtue of the formation of organic acids, which can form soluble and absorbable ligands with the minerals, thereby preventing the formation of insoluble complexes with for example phytic acid (Rousseau et al., 2020).

Fermentation is a metabolic process in which sugars are oxidized to produce energy; it also improves the absorption of minerals from the plant-based foods (Samtiya et al., 2020). Fermentation shows reduce in antinutritional factors, positive effects on protein digestibility, texture and aroma, and improve biological value of foods (Devi et al., 2019). Fermentation is such an important process, which significantly lowers the content of anti-nutrients such as phytic acid, tannins, and polyphenols of cereals. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate, which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc, calcium several folds (Samtiya et al., 2020). The combination of soaking, sprouting, cooking and fermentation appeared to have beneficial effects as methods of processing. It improved the nutrient quality and reduced the anti-nutritional factors inherent in sprouted cereal products to safe levels (Devi et al., 2019). One study reported that when germinated millets sprouts were fermented at 30 °C with mixtures of probiotics culture consisting of *Saccharomyces diasticus*, *Saccharomyces cerevisiae*, *Lactobacillus brevis* and *Lactobacillus fermentum* for 72 h, approx. 88.3% reduction of phytic acid content was observed (Samtiya et al., 2020).

### 3. ROLE OF FORTIFICATION IN ENHANCING IRON BIOAVAILABILITY

The need and consumption of fortified foods have increased significantly in recent years, there is great concern about the nutritional aspects and processed foods products consumed in the daily diet, this is due to the high nutritional deficiencies in the world population (Purizaca-Santisteban et al., 2023). Fortification is the common practice for increasing the content of an essential micronutrient like vitamins and minerals in foods, to improve the nutritional quality of the food, and to provide public health benefits at minimal risk (Shubham et al., 2020). Food fortification offers a more cost-effective approach in providing additional iron to most segments of the population by mass fortification of staples such as wheat and maize flour, or condiments such as salt, fish sauce, or soy sauce (Hurrell et al., 2004). Iron deficiency arises when physiological demands are not met because of scarce ingestion, uptake or usage, or ample Fe dissipations (Gharibzahedi & Jafari, 2017). A major criterion in deciding the best way of delivering micronutrients either by fortification or supplementation depends on the target population group (Shubham et al., 2020).

In practice, a small number of iron compounds are more regularly used for food fortification. These include ferrous sulphate, ferrous gluconate, ferrous fumarate, ferric pyrophosphate (FPP), sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), ferrous bisglycinate (FBG), and elemental iron powders. NaFeEDTA was chosen as the fortificants because it appeared to counteract the inhibitory effect of phytate observed on  $\text{FeSO}_4$ -fortified bread which was reported in a human study (Ologunde et al., 1994). Food fortification offers a more cost-effective approach to providing additional iron to most segments of the population by mass fortification of staples such as wheat and maize flour, or condiments such as salt, fish sauce, or soy sauce (Hurrell et al., 2004). Regular consumption of fortified staple foods aids in the consistent supply of nutrients without impediments caused from seasonal availability of foods. Veritably, widely consumed foods when employed as a vehicle for fortification can potentially augment the nutritional status of a huge population in a simple, efficacious, and economical way. This is known as “mass” or “universal fortification” (Kaur et al., 2022). A Brazilian study reported that iron consumption from biscuits made with iron-fortified flour accounted for 20%–30% of the recommended daily intake (RDI) for iron in children, 9.5%–14% in adults and 5%–7.2% in pregnant women (Perera et al., 2023). One study reported that addition of iron and zinc fortificants to wheat flour did not exhibit any critical alteration in the basic composition of the flours, however, marginal differences in various flour attributes concerning rheology were detected (Ahmed et al., 2012). Recently, scientists have worked out a newer food-based strategy termed as Food-to-food fortification (FtFF). This strategy involves fortification of staple food vehicles with food-based fortifiers that are economical and locally available to the target population. This emerging strategy involves the incorporation of nutrient dense food as a fortifier in a staple food vehicle to enhance its micronutrient content. Additionally, FtFF leverages the utilisation of crops that have been marginalised due to overproduction of staple crops; and to develop local value-added food enterprises which may potentially promote the fiscal development of community besides providing health and nutritional benefits (Kaur et al., 2022).

On comparing food supplementation, fortification, and dietary diversification, food fortification is known to be cost effective (cost of \$66 per Disability-Adjusted Life Year - DALY), while supplementation and dietary diversification had a cost of \$179 and \$103 per DALY, respectively (Shubham et al., 2020). Another study in the Philippines revealed that a fruit powder beverage fortified with iron and iodine (4.8mg iron and 48mg iodine per 25g beverage powder) was advantageous to school-aged children (1–6 grades) with iron and iodine deficiencies. The subjects demonstrated significant improvements in iron storage, iodine status, cognitive ability and physical fitness after consuming 200mL of this beverage twice daily for 16 weeks (Perera et al., 2023). Therefore, for effective implementation of the fortification process, more studies are required, considering public awareness and acceptability (Shubham et al., 2020).



#### 4. EFFECTS OF ANTINUTRITIONAL FACTORS ON IRON BIOAVAILABILITY

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients, in this way they reduce the nutrient bioaccessibility and bioavailability from food (Rousseau et al., 2020). The antinutrients such as lectins, glucosinolates, phytates, oxalates, or tannins among others emerge because of defence mechanisms with which plants protect themselves from the nearby conditions. Antinutrients are plant compounds which have traditionally been considered harmful to health due to their potential to limit the bioavailability of essential nutrients (Table 2) (López-Moreno et al., 2022). When used at low levels, phytic acid, lectins and phenolic compounds as well as enzyme inhibitors and saponins have been shown to reduce blood glucose and/or plasma cholesterol and triacylglycerols. Furthermore, saponins are reported to act effectively in maintaining liver function, preventing osteoporosis as well as platelet agglutination (Popova & Mihaylova, 2019). Many in vitro and in vivo considerations have stated the negative effects of dietary fibres, phytic acid, polyphenols and on iron bioavailability. Since, these compounds specifically can interfere with the absorption of minerals, they are generally referred to as mineral antinutrients (Rousseau et al., 2020).

**Table 2. Impact of Various Antinutritional Components on Iron Bioavailability.**

Author/Year	Nutrients	Objectives	Limitations	Nutritional Consequences
Baye et al. (2017)	Dietary Fibre	To assess the impact of dietary fiber on absorption of minerals	High fiber intake can reduce iron absorption when not balanced with enhancers	Foods or diets high in dietary fibre may alter mineral metabolism, phytate normally present in fibrous foods is the primary cause of alteration of mineral metabolism, rather than the fibre itself.
Rousseau et al. (2020)	Phytate	To assess how phytate affects the absorption of iron	Requires processing methods like soaking or fermentation to reduce its impact	High consumption triggers Fe absorption. Zn, Fe, Ca and probably Mg are poorly absorbed.
Abera Teka (2019)	Polyphenol	To investigate the impact of polyphenols on iron absorption	Present in commonly consumed beverages like tea and coffee, affecting dietary iron intake	They disrupt the digestibility of proteins. Reduce the digestibility of starch, protein and lipids. It inhibits non-heme-Fe absorption.
Devi et al. (2019); Popova & Mihaylova (2019)	Tannins	To examine how tannins hinder the absorption of minerals	Found in a wide range of plant-based meals and drinks, which makes managing a nutritious diet challenging.	Reduces Fe bioavailability and/or iron status. Inhibits non-heme-Fe absorption. Free radical scavenger and activates antioxidant enzymes.
Devi et al. (2019)	Lectins	To understand the role of lectins in nutrient metabolism	Nutrient scarcity and digestive problems might result from high amounts.	Interfere with hormone balance and deplete nutrient reserves leading to severe growth inhibition.

This table summarizes the impact of various dietary components on iron absorption, highlighting key findings and associated limitations.

#### 4.1. Dietary Fibre

The term dietary fibre was originally coined by Hipsley (1953) and referred to as the non-digestible components of the plant cell wall (Baye et al., 2017). Dietary fibre was conventionally termed as the non-digestible mechanisms of the plant cell wall. Most recently, dietary fibre is described as “edible carbohydrate monomers (naturally occurring in foods, as well as isolated, modified and synthetic polymers with proven physiologic effects of benefit to health) with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of human” (Rousseau et al., 2020). Several *in vitro* studies have shown that semi-purified insoluble (cellulose, hemicelluloses and lignin) as well as soluble fibres (gums and pectin) have mineral-binding properties (Baye et al., 2017). The stimulation of dietary fibre in the diet often incorporates the use of wheat bran. When wheat bran was added to a meal the absorption of non-haem-Fe from all foods tested was impaired. The non-haem-Fe may comprise 100% of the total Fe intake; thus, it is the primary source of absorbable Fe. The inhibitory effect of bran on Fe absorption may be attributed primarily to its phytate rather than its dietary fibre content. However, if meat (or fish) or ascorbic acid, or both, are present, the binding properties of the phytate in the bran may be lessened or overcome entirely (Harland, 1989). The impact on mineral bioavailability of fibres depends on individual characteristics of the fibre. For example, the solubility of the fibre plays an important role (Rousseau et al., 2020). Several dietary factors may affect the nutrient bioavailability of plant foods when they are consumed, including: (1) the chemical form of the nutrient in the food and the nature of the food matrix; (2) interactions occurring between nutrients and other organic components within the plant food; (3) pretreatment of the food during processing and/or preparation (Gibson et al., 2006).

Studies investigating the correlation between iron bioavailability and dietary fibers have not demonstrated results that agree with each other. Insoluble dietary fibers are known to inhibit mineral bioavailability. However, soluble dietary fibers have a smaller effect on intestinal iron absorption. One human study was conducted on the effect of a prebiotic mixture composed of soluble fibers (inulin, polio dextrose, arabic gum, and guar gum) on heme nonheme iron. They found that while the prebiotic mix improved the heme iron absorption, nonheme iron absorption was unaffected (Piskin et al., 2022).

#### 4.2. Phytates

Phytate is an organic compound (myo-inositol hexaphosphate) which occurs in all plants and serves as the storage form of Phosphorous (P) in the living plant (Harland, 1989). Phytate is formed during the maturation stage of the plant seed and contains 60–90% of the total phosphorous content in dormant seeds, where it is accumulated as mixed salts of several cations, including iron, zinc, magnesium, calcium and potassium (Rousseau et al., 2020). Phytase is the enzyme which hydrolyses phytate, thereby releasing the bound mineral or minerals. Phytate is a potent chelator of minerals and, thus, its presence in a food will strongly dictate the outcome of minerals associated with this molecule (Harland, 1989). Also, phytates can form complexes with proteins, however, this interaction is dependent on pH, isoelectric point, ionic strength and amino acid availability (López-Moreno et al., 2022). Besides its role in mineral bioavailability, phytate can also influence protein digestibility. This complex formation with proteins alters the protein structure, which make the proteins less soluble, and affects the enzymatic degradation and peptic digestion (Rousseau et al., 2020). A net positive charge (pH < isoelectric point) seems to be necessary for the formation and stability of phytate-protein complexes (Wang & Guo, 2021).

It has also been postulated that regular consumption of phytate can trigger an inhibition of the negative effect of this compound on iron absorption in women with suboptimal iron store, a phenomenon known as phytate adaptation (López-Moreno et al., 2022). In addition, one study showed that iron absorption in rats decreased significantly when the molar ratios of phytic acid to iron were above 14:1. For example, 100 g beans (*Phaseolus vulgaris*) can contain up to 2.38 g phytic acid and up to 14 mg iron (dry weight), this means that in realistic cases the ratio exceeds 14:1 (Rousseau et al., 2020). Another study reviewed the evidence from human studies investigating the impact of phytase on iron and zinc bioavailability. They concluded that phytase promotes the absorption of iron and zinc from phytate-rich meals and can potentially improve magnesium, calcium, and phosphorus absorption (Piskin et al., 2022).

#### 4.3. Polyphenols

Polyphenols are an integral part of the human diet since they are omnipresent in plant tissues, where they serve as protective substances against harmful external influences (e.g. Ultraviolet radiation and aggression by pathogens). Phenolic compounds are composed of an aromatic ring with at least one hydroxyl group in their structure. In plants, most polyphenols are conjugated to one or two sugar moieties (Rousseau et al., 2020). Polyphenols are found in the human diet mainly due to their presence in vegetables, cereals, spices, tea, coffee, red wine, and cocoa. Polyphenols are known iron bioavailability inhibitors and are assumed to work similarly to phytate by forming a complex with iron (Piskin et al., 2022). It is proposed that polyphenols can affect iron status by regulating expression and activity of proteins involved in either the systemic regulation of iron metabolism or iron absorption (Lesjak, 2015). During digestion, the phenolic compounds from the food or beverage released and can form complex with Fe in the intestinal lumen making it unavailable for absorption. Nearly all beverages reduced iron absorption depending on the amount of total polyphenols (Abera Tekla, 2019).

Ultimately, polyphenols are proven to be the most abundant antioxidants in the human diet (Rousseau et al., 2020). One study investigated the effect of bean polyphenols on iron absorption in humans to determine the effect of bean polyphenols

on iron absorption in the absence of phytic acid, increasing quantities of red bean hulls were introduced as a source of bean polyphenols to non-inhibitory bread meals where phytic acid had been destroyed during dough fermentation. In this study it was observed that, 50 mg and 200 mg of bean polyphenols lowered iron bioavailability by 18% and 45%, respectively, demonstrating that red bean polyphenols inhibited iron bioavailability in a dose-dependent manner (Piskin et al., 2022). Another recent study reported that bioavailability of iron from NaFeEDTA when added to wheat flour-based meals in both nonanemic women and women with IDA when consumed with and without traditional Moroccan green tea, which had 492 mg GA/ml polyphenols per meal portion serving size/day. The results showed that tea consumption reduced iron absorption from NaFeEDTA by more than 85% in both IDA and nonanemic women (Piskin et al., 2022). Furthermore, few studies reported that there is a beneficial effect of beef on non-heme Fe absorption from a meal rich in phytate and polyphenol compounds. This study on the influence of beef on Fe bioavailability from white kidney bean seeds ragout, commonly consumed in Tunisia, is of great importance in improving the level of Fe bioavailability found in this meal (Hamdaoui et al., 2003).

#### 4.4. Tannins

Tannins are phenolic compounds, which consist of molecular weights greater than 500 Da. One of the properties of these compounds is that they can precipitate proteins (Samtiya et al., 2020). Tannins are grouped in two categories: (1) hydrolysable tannins, and (2) condensed tannins. However, hydrolysable tannins are present in trace amounts while the condensed tannins are more predominant in commonly consumed foods (Devi et al., 2019). Tannins exhibit antinutritional properties by impairing the digestion of various nutrients and preventing the body from absorbing beneficial bioavailable substances (Popova & Mihaylova, 2019). The tannins also have some health benefits due to their antioxidant properties and considered as cardio-protective, anti-carcinogenic, anti-inflammatory and anti-mutagenic. They act as free radical scavengers and also activates the antioxidative enzymes (Devi et al., 2019). Tannins can also bind and shrink proteins. Tannin-protein complexes may cause digestive enzymes inactivation and protein digestibility reduction caused by protein substrate and ionisable iron interaction (Popova & Mihaylova, 2019).

The majority studies isolating the influence of tannin consumption over time without confounding antinutritional factors have been done in animals. Studies supporting reductions in iron bioavailability and/or iron status in animal models have typically employed use of tannic acid or tea. The consumption of 100g/L green tea polyphenols compared to water consumption in rats over eight weeks, resulted in a significant reduction in hepatic iron and haemoglobin (25% and 10%, respectively (Delimont et al., 2017). Most tannin research relates to impaired haem-iron bioavailability. Tannins in both tea and coffee have been shown to bind Fe by forming insoluble iron tannates within the human gastrointestinal tract (Harland, 1989). Another study reported that 4-week haemoglobin depletion repletion in piglets observed that consumed meals with significantly different tannin amounts from red or white bean feed found no differences in haemoglobin, haemoglobin repletion-efficiency (haemoglobin replaced per iron intake), or weight gain at endpoint (Delimont et al., 2017).

#### 4.5. Lectins

The word lectin derived from the Latin word “legere” which means “to select”. Lectins are a type of sugar, or carbohydrates binding protein and form the “glyco” portion of glycoconjugates (glycoproteins) of the cell membranes (Devi et al., 2019). They have the ability to bind, without modifying, to either carbohydrates or glycoconjugates (glycoproteins, glycolipids, polysaccharides) (Popova & Mihaylova, 2019). Plant lectins are found in nuts, cereals and mainly in the seeds of leguminous (López-Moreno et al., 2022). When large quantities of lectins are introduced in the body, the gut wall develops holes, and intestinal permeability, causing the leaky gut syndrome. Lectins can make cells act as if they have been stimulated by insulin or cause the insulin release by the pancreas. Lectins can also cause autoimmune diseases by presenting wrong immune system codes and stimulating the growth of some white blood cells (Popova & Mihaylova, 2019). Lectins are toxic and can also interfere with hormone balance and deplete nutrient reserves leading to severe growth inhibition. However, lectins may be beneficial by stimulating gut function, preventing tumour growth and curing obesity (Devi et al., 2019). Phytohemagglutinin is a tetrameric glycoprotein with a molecular mass of 120 kDa, which is found in kidney beans and consists of two diverse subunits. In rats, kidney bean phytohemagglutinin appears to upregulate the function and metabolism of the whole gastrointestinal tract, which includes growth of the small intestine, increased length of the tissue and number of intestinal crypt cells. Purified lectins from beans or soybeans impaired rat growth, induced enlargement of the small intestine, caused damage to the epithelium of the small intestine, and stimulated hypertrophy and hyperplasia of the pancreas (Samtiya et al., 2020).

### 5. CONCEPTUALIZATION AND REVIEW FRAMEWORK

#### 5.1. Identification of Research Questions

The primary objective of this scoping review aims to explore the impact of food processing, fortification, and antinutritional factors on iron bioavailability. The research questions (RQ) guiding this review are: Firstly, **RQ1:** How do different food processing techniques influence iron bioavailability in various dietary sources, Secondly, **RQ2:** What is the combined effect of food processing techniques and fortification on iron bioavailability in staple foods, Lastly, **RQ3:** How do antinutritional



factors such as phytates, polyphenols, and tannins impact iron absorption, and what strategies can mitigate their effects.

### 5.2. Search Strategy & Screening

A comprehensive search strategy was implemented to identify relevant studies from major academic databases, including PubMed, Elsevier, Scopus, Web of Science, and MDPI. Only peer-reviewed original research articles published in English were included. No specific outcome-related keywords were used to ensure a broad selection of studies. Screening was conducted based on titles, abstracts, and full-text evaluations. The inclusion criteria required studies to be published between 2010 and 2025, focusing on food processing techniques, iron fortification, or antinutritional factors affecting iron absorption. Studies lacking relevant data, those published in non-peer-reviewed sources, or articles not in English were excluded. After initial screening, 113 studies were identified, and following the application of inclusion and exclusion criteria, 40 studies were selected for final analysis.

### 5.3. Applying Eligibility Criteria

After removing 59 duplicate records, 113 studies remained for screening. During this phase, 29 studies were excluded due to the following reasons:

- • Irrelevant topic (n = 10)
- • Insufficient data or incomplete studies (n = 11)
- • Non-English articles (n = 8)

A total of 84 full-text articles were further assessed for eligibility, leading to the exclusion of 44 additional studies due to:

- • Repetition of information from already included literature (n = 25)
- • Lack of detail for adequate evaluation (n = 11)
- • Irrelevant study outcomes (n = 5)

Ultimately, 40 studies met all eligibility criteria and were included in the final review.

### 5.4. Inclusion and Exclusion Criteria

#### Inclusion Criteria:

- Research evaluating the effectiveness of iron fortification strategies in improving absorption.
- Studies analysing the effects of antinutritional factors (phytates, polyphenols, tannins, etc.) on iron absorption and mitigation techniques.

#### Exclusion Criteria:

- Research focusing solely on traditional food processing methods without evaluating iron bioavailability.
- Articles published in non-peer-reviewed sources or grey literature without rigorous validation.

## 6. RESULTS

The current study follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines for reporting systematic reviews (Page et al., 2021). It was divided into four steps: identification, screening, eligibility, and inclusion. Initially, 125 articles were gathered from reputable and widely recognized repositories/databases, including PubMed, Elsevier, MDPI, Scopus, and Web of Science. Additionally, 47 records were identified from other sources. After removing 59 duplicate records, 113 articles were screened based on their titles and keywords. During the screening phase, 29 articles were excluded due to irrelevant topics (10 studies), insufficient or incomplete data (11 studies), and non-English language (8 studies). A total of 84 full-text articles were assessed for eligibility. Following this assessment, 44 articles were excluded due to repetition of information from other included literature (25 studies), lack of sufficient detail for adequate evaluation (11 studies), and non-relevant outcomes (5 studies). Ultimately, 40 studies were deemed suitable for inclusion in the final analysis. The PRISMA flowchart is given below in Figure 1.

This section addresses the research questions as follows: **(RQ1)** the influence of food processing techniques on iron bioavailability in various dietary sources, **(RQ2)** the role of combined effect of food processing techniques and fortification on iron bioavailability in staple foods, and **(RQ3)** the impact of antinutritional factors such as phytates, polyphenols, and tannins on iron absorption, along with strategies to mitigate their effects.

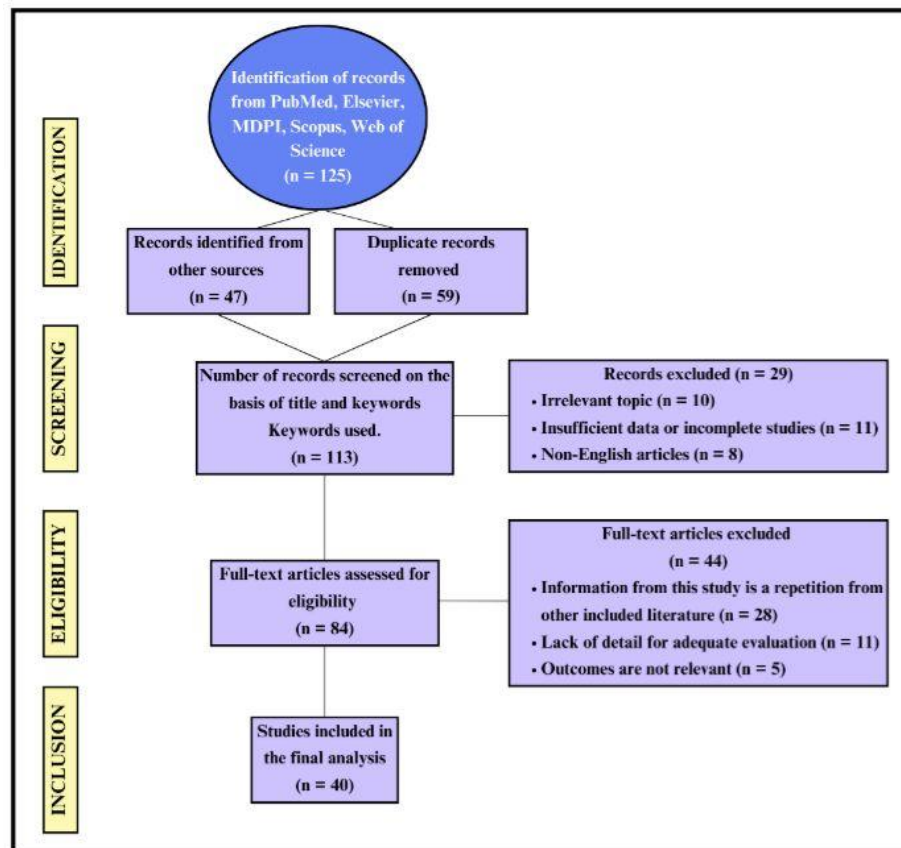


Figure 1. Data collection based on PRISMA chart.

### 6.1. Food Processing and Iron Bioavailability

**RQ1:** How do different food processing techniques influence iron bioavailability in various dietary sources?

Piskin et al. (2022) and Sulaiman et al. (2021) reported that food processing methods, such as fermentation, germination, soaking, and heat treatments, play a significant role in improving iron bioavailability. These techniques can reduce antinutritional factors that inhibit iron absorption, thereby enhancing its bioaccessibility. For instance, fermentation of cereals and legumes degrades phytates, increasing non-heme iron absorption, while cooking and mechanical processing improve the release of iron from plant matrices, making it more available for uptake.

### 6.2. Iron Bioavailability in Staple Foods

**RQ2:** What is the role the combined effect of food processing techniques and fortification on iron bioavailability in staple foods?

Kaur et al. (2022) and Piskin et al. (2022) suggested food processing techniques like fermentation, germination, and thermal processing can reduce antinutrients (e.g., phytates), enhancing iron bioavailability in staple foods. Fortification with bioavailable iron compounds further improves absorption. The combined approach ensures better dietary iron intake, addressing deficiencies effectively. Optimal processing methods maximize fortification benefits, making staple foods more nutritionally adequate for populations at risk of iron deficiency.

### 6.3. Antinutritional Factors and Their Mitigation

**RQ3:** How do antinutritional factors such as phytates, polyphenols, and tannins impact iron absorption, and what strategies can mitigate their effects?

Ram et al. (2020) reported that antinutritional compounds like phytates, polyphenols, and tannins inhibit iron absorption by forming insoluble complexes with iron. Strategies to mitigate their effects include food processing techniques such as soaking, sprouting, and fermentation, which degrade these inhibitors. Additionally, dietary modifications like pairing iron-rich foods with enhancers such as vitamin C and avoiding tea or coffee with meals can improve iron absorption. Combining multiple strategies ensures better iron bioavailability and supports nutritional interventions for iron deficiency.

## 7. CONCLUSION

Iron bioavailability is influenced by dietary components, food processing, and fortification. Food processing methods like soaking, fermentation, and thermal treatments help reduce phytates, polyphenols, and tannins, enhancing iron solubility. Fortification is crucial in addressing iron deficiency, but its effectiveness depends on the iron compound used and the presence of enhancers like ascorbic acid. Antinutritional factors in plant-based foods remain a challenge, as they form insoluble iron complexes. Optimizing food formulations to minimize inhibitors while incorporating enhancers is essential. Future research should refine processing and fortification techniques to maximize absorption without compromising food quality. Public health strategies must consider dietary habits to ensure effectiveness. A holistic approach combining food technology, nutrition, and policy is key to combating iron deficiency. Advancing science-driven solutions can improve iron intake and absorption, reducing deficiency-related health risks worldwide.

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