The Pharma Innovation Journal 2022; 11(7): 1061-1069

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 1061-1069

www.thepharmajournal.com
Received: 01-04-2022
Accepted: 05-05-2022

Simranjeet Kaur
M.Sc. Food Science and Technology, Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Corresponding Author:
Simranjeet Kaur
M.Sc. Food Science and Technology, Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Ferritin core nanoparticulate: A source of iron fortification

Simranjeet Kaur

Abstract
To keep serum iron levels stable in the human body by recycling damaged erythrocytes and red blood cells, dietary iron is absorbed by duodenum enterocytes and the proximal jejunum before being absorbed in the serum. Traditional definitions of ID, which rely on low serum ferritin or iron levels as diagnostic thresholds, fail to account for changes in these parameters caused by chronic inflammation. The treatment of iron-deficiency anaemia includes addressing the underlying cause, such as gastrointestinal bleeding, as well as taking iron orally. To improve absorption, iron supplements should be taken without food. Treatment often produces a rapid response in 14 days. Although intravenous iron is more consistently and quickly delivered to the reticuloendothelial system than oral iron, it does not result in a faster increase in haemoglobin levels. Food fortification is a long-term and cost- effective technique for preventing iron deficiency anaemia. Iron supplements, on the other hand, frequently cause unwanted organoleptic changes and limit food acceptance and use. As a result, the fundamental difficulty in developing iron-fortified foods is to insert iron fortification more effectively in food to boost iron absorption without affecting the sensory features. It is an iron storage protein that consists of a spherical polypeptide shell which encloses a 6 nm core of the inorganic ferrihydrite hydrochloride, different ferritins have different amino acid sequences, but they all have the same structure. Spherical proteins consist of 24 subunits of mass 450, 500 kDa and a diameter of about 12 nm and an inner cavity of about 8 nm are part of the ferritin family. In the treatment of infectious diseases of the intestine, this mechanism is also promising. The iron in the protein shell does not touch the food ingredients and/or digestive tract cells. The food should be safe from oxidation or sensory alteration. In addition, oxidative damage can also be limited to intestine cells. The slow release of iron from the ferritin protects the cells of other iron supplements against oxidative damage. There are other difficulties with the production of bioactive food with high ferritin iron content. The concentration of ferritin iron in food of plant origin varies with the botany of the seeds or grains used. However, it also results from crop cultivation conditions and food processing. Since ferritin is a protein, food should be processed with non-thermal denaturation conditions. The method of iron speciation in plant samples was shown to have survived some thermal treatment, although the temperatures were above those which were known to degrade protein.

Keywords: Ferritin core nanoparticulate, iron fortification, traditional definitions

1. Introduction
Many physiological functions, including erythropoiesis, circulatory oxygen delivery and transfer, growth, development, and antioxidant capability of many organs, require iron. Ferric iron (Fe3+) and ferrous iron (Fe2+) are the most frequent types of iron existing in the human body (Brissot *et al*., 2018) [1]. To keep serum iron levels stable in the human body by recycling damaged erythrocytes and red blood cells, whereas dietary iron is absorbed by duodenum enterocytes and the proximal jejunum before being absorbed in the serum (Ganz and Nemeth., 2012). The heart, as a high-energy-consuming organ, has stringent iron homeostasis criteria. For example, adequate iron is required for oxidative phosphorylation and redox signalling, however, iron overload can result in excessive reactive oxygen species (ROS) formation, lipid peroxidation, and organ damage through the Fenton reaction (Drakesmith *et al*., 2015) [3]. Iron deficiency (ID) with or without associated anaemia is relatively common in chronic diseases with inflammatory aspects, such as obesity. Traditional definitions of ID, which rely on low serum ferritin or iron levels as diagnostic thresholds, fail to account for changes in these parameters caused by chronic inflammation (Ramakrishnan *et al*., 2015) [4]. The treatment of iron- deficiency anaemia includes addressing the underlying cause, such as gastrointestinal bleeding, as well as taking iron orally. To improve absorption, iron supplements should be taken without food. Iron absorption is aided by a low gastric pH. Treatment often produces a rapid response in 14 days.

It is characterised by an increase in haemoglobin levels. Iron supplementation is required for at least three months to replenish tissue iron stores and should continue for at least one month after haemoglobin levels have returned to normal (Anderson *et al*., 2013) [5]. Ferrous sulphate is a low-cost and effective therapy that is typically administered in two to three divided doses per day. Constipation, nausea, decreased appetite, and diarrhoea is all side effects of taking iron orally. If the patient is intolerant to oral iron, has malabsorption such as celiac disease, post-gastrectomy, or achlorhydria, or the losses are too great for oral therapy, intravenous iron may be required. Although intravenous iron is more consistently and quickly delivered to the reticuloendothelial system than oral iron, it does not result in a faster increase in haemoglobin levels (Berdoukas *et al*., 2015) [6].

Food fortification is a long-term and cost-effective technique for preventing iron deficiency anaemia (Lakhal *et al*., 2015) [12]. Iron supplements, on the other hand, frequently cause unwanted organoleptic changes and limit food acceptance and use (Triner *et al*., 2017) [7]. As a result, the fundamental difficulty in developing iron-fortified foods is to insert iron fortification more effectively in food to boost iron absorption without affecting the sensory features (Xue *et al*., 2014) [8]. The addition of Mg or Ca to ferric oxide-based nano-structured compounds boosted solubility and contributed to improved iron fortification. They are highly soluble in dilute acid and are likely to be absorbed effectively in the gut without causing significant colour changes (Qiao *et al*., 2012) [9]. Various forms of iron nanoparticles, such as zero-valent iron nanoparticles, iron phosphate nanoparticles (FePO4 NPs), and magnetite nanoparticles (Fe3O4 NPs), have recently been used to replace commonly used iron salt in food fortification due to their high biocompatibility and toxicological safety (Mancias *et al*., 2015). However, when utilized alone, these iron nanoparticles oxidize quickly and agglomerate, limiting their applicability in food systems (Ross *et al*., 2012) [11].

Ferritin was first discovered by Laufberger in the year 1937. It is an iron storage protein that consists of a spherical polypeptide shell (Apoferritin) which encloses a 6 nm core of the inorganic ferrihydrite hydrochloride (5Fe2O3·9H2O), different ferritins have different amino acid sequences, but they all have the same structure. Spherical proteins consist of 24 subunits of mass 450, 500 kDa and a diameter of about 12 nm and an inner cavity of about 8 nm are part of the ferritin family (Vannucci *et al*., 2015). In the treatment of infectious diseases of the intestine, this mechanism is also promising. The iron in the protein shell does not touch the food ingredients and/or digestive tract cells. The food should be safe from oxidation or sensory alteration (Hosny., 2015). In addition, oxidative damage can also be limited to intestine cells. The slow release of iron from the ferritin protects the cells of other iron supplements against oxidative damage. There are other difficulties with the production of bioactive food with high ferritin iron content. The concentration of ferritin iron in food of plant origin varies with the botany of the seeds or grains used. However, it also results from crop cultivation conditions (especially soil iron concentration) and food processing (Izquierdo., 2016). Since ferritin is a protein, food should be processed with non-thermal denaturation conditions. Therefore, the temperature or the exposure period should be restricted to elevated temperatures. The method of iron speciation in plant samples was shown to have survived some thermal treatment, although the temperatures were above those which were known to degrade protein, i.e. more than 80°C. Samples spike with labelling ferritin bean and mass ionisation of thermal ionisation spectrometry isotope dilution (IDMS) enables ferritin iron quantification. The colourimetric analysis of the various iron shapes taken from food samples, organically and inorganically complex as well as the ionic form of iron are another approach that is worth considering (Zhang *et al*., 2015).

Nanoparticles (NPS) are a wide class of materials containing at least one dimension of particulate matter less than 100 nm (Lui *et al*., 2018). NPS are not simple molecules alone and therefore consist of three layers, i.e. (a) the surface layer that can operate with various molecules, metal ions, surfactants, and polymers. (b) Shell Layer that, in all respects, is chemically different from the core material and (c) the core is basically the central part of the NP and refers generally to the NP itself (Kornienko *et al*., 2018). Also, recently ferritin has been used to produce nanoparticles for electronics with conduction and magnetic properties based on magnesium, cobalt, and copper (Wang *et al*., 2018). But the regulation of sizes and mineral loads is becoming increasingly clear to manufacture nanomaterials like memory devices and for fluorescent labelling of biomolecules (Chen *et al*., 2018).

2. Iron deficiency anaemia

Iron deficiency anaemia is the most common and easily treated of all anaemias. There is a growing understanding that iron deficiency can cause symptoms unrelated to anaemia and can be linked to a variety of diseases (Evstatiev and Gasche., 2012) [13]

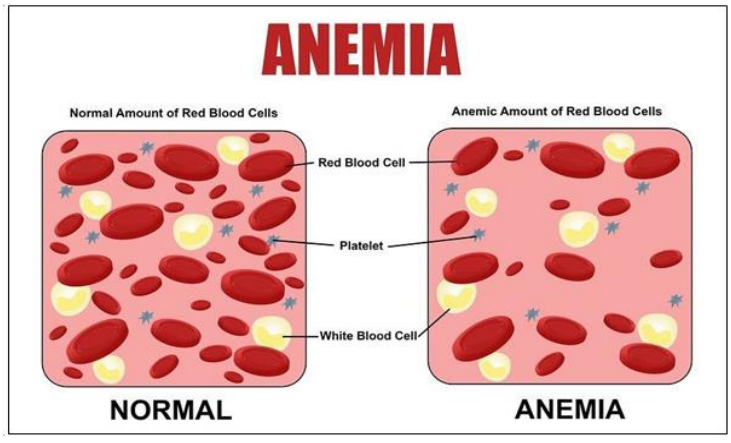

Fig 1: Difference between normal red blood cells and anaemic amount in red blood cells

2.1 Iron metabolism

Iron can be found in a variety of foods, with meat being the most abundant source. Iron in food is mostly in the ferric form (Fe3+), which is reduced to ferrous by stomach acid (Fe2+). Two receptors on the mucosal cells of the jejunum absorb iron (Levi *et al*., 2016) [23]. One receptor is specific for heme-bound iron and absorbs 30-40% of ingested heme iron. The other receptor, divalent metal transporter (DMT1), also absorbs inorganic iron but is 1 to 10% less efficient. Iron is exported from the enterocyte by ferroportin and delivered to plasma transferrin, the primary iron transport molecule. Transferrin, by binding to the transferrin receptor on the red cell membrane, can deliver iron to the marrow for use in red cell production or to the liver for storage. Ferritin is an iron storage protein (Porwal *et al*., 2012) [24]. The ferritin protein is made up of 24 ferritin subunits that work together to form a shell that can hold up to 4500 iron molecules (Evstatiev and Gasche., 2016). Iron from senescent red cells' haemoglobin is recycled by binding to ferritin in the macrophage. It is then either transferred to transferrin for recycling into developing red cells or stored. This system is extremely efficient, losing less than 5% of the iron in total red cell mass. Hepcidin, a protein, regulates iron absorption and iron release from the store (Hudak *et al*., 2016) [14, 26]. Hepcidin binds to ferroportin, causing it to degrade. Iron cannot be released from enterocytes or hepatocytes when hepcidin degrades ferroportin, resulting in both a lack of iron absorption and a halt in iron release to developing red cells. Not only does iron stimulate hepcidin synthesis, but so does inflammation (Pavord *et al*., 2012) [25]. Hypoxia increased erythropoiesis, and iron deficiency is all lower levels. Men should consume 8 mg of iron per day, while premenopausal women should consume 18 mg, increasing to 27 mg per day during pregnancy (Hershko and Camaschella., 2014) [27]. Meats are high in heme iron, which allows iron to be absorbed much more effectively. Nonmeat iron sources have lower iron stores, are less effectively absorbed, and are difficult to consume in sufficient quantities to meet iron requirements (Hudak *et al*., 2016) [14, 26]

2.1.1 Symptoms

Iron deficiency can cause symptoms due to both the lack of iron and the resulting anaemia. Anaemia symptoms include fatigue, tachycardia, and a lack of endurance. Pica, or strange cravings for ice or clay, can occur (Hershko and Camaschella., 2014) [27]. Symptoms in iron- depleted but nonanemic patients are becoming more widely recognised. Given the importance of iron in many cellular proteins and enzymes, particularly cytochromes and myoglobin, it is not surprising that symptoms can appear before anaemia. The most severe symptom is fatigue, which can occur even with minor reductions in iron stores (Weng *et al*., 2015) [28]. Two studies show that oral iron supplementation improves fatigue in iron-deficient but not anaemic women with ferritins less than 50 ng/mL (Vaucher *et al*., 2012) [16] while a third study shows that parenteral iron improves fatigue in women with ferritins less than 15 or iron saturation less than 20% (Sharma *et al*., 2016) [17]. Another recent study found that iron supplementation improved fatigue in iron-deficient but nonanemic fatigued adolescents (Leclercq *et al*., 2014) [18]. These studies, along with the previously mentioned improvements in athletic performance, clearly demonstrate the role of iron in well-being that extends beyond haemoglobin levels. Cold intolerance is another common symptom of iron deficiency (Von *et al*., 2015) [19]. Some of these symptoms are caused by anaemia, and they usually go away when the anaemia is treated. However, some patients with nonanemic iron deficiency experience cold intolerance. This could be due to a decrease in thyroid hormone efficacy. Because the effectiveness of the intracellular thyroid is dependent on iron stores, a lack of iron reduces the hormone's effectiveness (Ruz *et al*., 2012) [29]. Patients with heart failure have a higher prevalence of both overt and functional iron deficiency. Iron deficiency can affect anywhere from 13% to 42% of people, with a higher percentage having functional iron deficiency (Qian *et al*., 2016) [20]. Replacing depleted iron stores has been shown to improve cardiac function and quality of life, as well as to decrease hospitalisation (Robinson *et al*., 2014) [21]. Patients with pulmonary hypertension are another group in which iron deficiency may be harmful. Iron chelation mimics hypoxia by upregulating genes that control the hypoxic response, so the cellular response to hypoxia is iron-dependent (Frise *et al*., 2016). Iron is important in the regulation of pulmonary vascular resistance. When compared to healthy controls, iron-deficient patients had an exaggerated hypoxic. DeLoughery rise in pulmonary artery pressures. Low iron stores have been observed in patients with pulmonary hypertension, and repletion has been found to lower pulmonary artery pressure (Buro *et al*., 2015). Restless leg syndrome is a condition in which patients experience leg cramps, unpleasant leg sensations, and the need to move their legs during the night. Many of these patients have low iron stores, as evidenced by studies of brain iron. Iron repletion alleviates symptoms in many patients, implying that low iron stores are pathogenic in at least some RLS patients (Moretti *et al*., 2015).

2.1.2 Treatment

Eating a diet rich in bioavailable iron can help treat iron deficiency, but it is unlikely to replenish iron stores on its own, though it can help maintain them. Meat is the iron-richest food. Cooking with iron skillets can also boost dietary iron, though the amount of iron enrichment varies (Rampton *et al*., 2014). Ferrous sulphate, taken three times a day, has long been used to treat iron deficiency. Recently, studies have suggested that lower doses of iron, such as 15 to 20 mg elemental iron daily, can be just as effective as higher doses and are better tolerated with fewer gastrointestinal side effects (Auerbach *et al*., 2016). A study comparing 15, 50, and 150 mg oral elemental iron found no difference in ferritin rise with any dose and less gastrointestinal toxicity with the smallest dose. Low-dose iron may be effective because enterocyte iron absorption appears to be saturable, and one dose of iron can "block" absorption of subsequent iron doses for the rest of the day (Avni *et al*., 2015). A recent study found an abrupt rise in hepcidin levels with iron consumption, highlighting this mechanism. Iron absorption can be increased by taking the iron supplement with meat protein (cow, pork, and fish) (Kim *et al*., 2014) [10]. Vitamin C aids absorption in a variety of ways. First, as a reducing agent, vitamin C aids in the retention of iron in its more soluble ferrous form. Second, iron and vitamin C can bind together to form a soluble complex. Concurrent calcium and fibre intake can reduce iron absorption, but this can be mitigated by taking vitamin C (Crabol *et al*., 2014). Importantly, tea is a powerful inhibitor of iron absorption; ingestion can reduce absorption by 90%.

Coffee also reduces iron absorption by roughly two-thirds the amount that tea does. A reasonable approach to oral iron replacement is, to begin with, a single ferrous sulphate taken with a meal that contains meat (if not vegetarian). Avoiding tea and coffee, as well as taking 500 mg of vitamin C with that meal, will help iron absorption (Zhu *et al*., 2010). If ferrous sulphate is not tolerated, ferrous gluconate, which contains less elemental iron per tablet, can be used instead. The reticulocyte count should increase in one week, and haemoglobin levels should begin to rise by the second week of therapy.

3. Iron fortification

Fortification is the common practice for increasing the content of an essential micronutrients like vitamins and minerals in foods, to improve the nutritional quality of the food, and to provide public health benefits at minimal risk (Bacanlı *et al*., 2015) [32]. The main aim of food fortification is to improve the nutritional content of the food. A major criterion in deciding the best way of delivering micronutrients either by fortification or supplementation depends on the target population group. Food fortification aids in easy access to achieving daily nutritional needs for rural communities without dependency on pharmaceutical supplements and has the potential to impact a large number of people cost-effectively. Iron supplementation involves the oral administration of pharmaceutical iron compounds while fortification of iron involves the delivery of iron through foods. Supplementation of iron can be practised when immediate action is required for increasing the level of iron in the human body as orally administered haem iron easily enters into the bloodstream (Chandrasekara *et al*., 2018) [31]. Whereas iron fortification helps target a specific group by supplying iron through foods that follow the regular absorption mechanism as that of the diet, resulting in a gradual increase in the iron status of the population. Although the effect of fortification of foods is not as quick as supplementation in targeting nutrient deficiency, fortification is an effective approach for sustainable benefits in the long run (Khan., 2015) [30]. This review provides unambiguous evidence that iron concentration in the human bloodstream can be improved through iron-fortified foods. Considering all age groups, the pooled positive intervention can result in reductions in anaemia and iron deficiency owing to the interference of fortified iron from the food in the various pathways of iron absorption. Although food fortification has several benefits, there exist certain limitations such as deterioration of the colour and flavour of food vehicles, determination of optimal delivery systems and the assurance of health impact and acceptability of biofortified foods (Ansari *et al*., 2017) [33]. However, fortification remains to be attractive in terms of cost, since the costs of conventional medications remain high, but fortified foods remain harder to reach the poorest; those who are most price sensitive. The study on the economics of fortification showed that the effectiveness of fortification in terms of cost ranges from $22 to
$60/day, remaining comparatively favourable to values of other healthcare interventions for children. Another study on the benefits of investment in iron fortification estimated a rise in benefit: cost ratio from 6:1 to 36:1, because of the combined effects of cognitive ability and physical productivity. In 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 (Bamzai and Bhat., 2015) [34]. Therefore, for effective implementation of the fortification process, more studies are required, considering public awareness and acceptability.

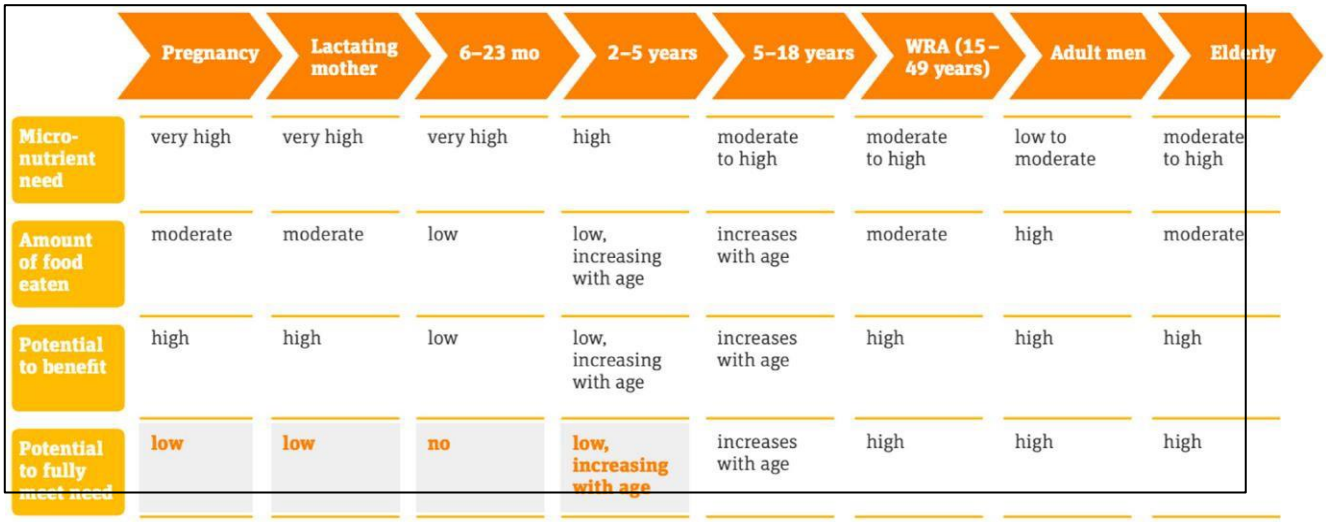

Fig 2: Benefits of food fortification across the life cycle

3.1 Different types of iron fortificants

3.1.1 Wheat and maize flours

Wheat and maize millers are equipped with the same processes (Zimmerman *et al*., 2018) [35]. The fortification method of the two flours is well and reasonably straightforward, as both food and micronutrient Premix are in powder form and fortified by wheat and maize flour for many decades. However, considerable development was needed in selecting sufficiently bioavailable iron compounds which do not trigger any sensory changes. The key sensory issue with cereal meals was iron-catalyzed oxidative rancidity that could be caused by soluble iron compounds such as iron sulfate, particularly in long-term flour storage under warm and moist conditions (Tsang *et al*., 2018) [36]. Unacceptable colour

reactions in iron-fortified cereal when fruit was added were also dangerous. The iron fortification compound for cereal meals was initially made of organoleptically inert elemental powders made from iron ferrous sulfate, ferrous fumarate, NaFeEDTA and electrolytic iron powder are the 4 iron fortification compounds recommended. Sensory tests, efficacy studies in women and children in LMIC (Low Middle Income Economies), the relative bioavailability of iron compounds, and approximate daily intake of iron were all found in the proposed fortification levels (Hackl *et al*., 2016) [37]. For high phytate wheat and corn flour, NaFeEDTA is the only WHO-recommended iron compound. The absorbance in NaFeEDTE-fortified wholegrain wheat rolls was 4 times higher than for iron, and in school foods, Indian children's iron status was improved by the NaFeEDTA-fortified wholegrain atta flour. (Diosady *et al*., 2019) [38].

3.1.2 Rice

As rice is mainly consumed as grain, it is technically more difficult to fortify rice than wheat or maize. Coating and extrusion are the two key rice fortification technologies, and they both involve the production of rice kernels that are micronutrient-fortified and then blended with rice kernels that are not fortifiable. Rice fortification has gained momentum in recent years with the advancement of fortified kernel technology (Cercamondi *et al*., 2016) [37, 39]. Extrusion may be done at various (cold, mild and hot) temperatures. Warm hot extrusion gelatinises the starch, partially or fully, to keep the kernel together and improve clarity and gloss, to look more precisely like non-fortified, equally clear rice grains. Cold extrusion involves a binder to hold the kernel and can be produced with special paste presses like warm extrusion (Namaste *et al*., 2017) [40, 41]. Hot extrusion involves a more costly, single or twin-screw extruder and additional investment in capital. A recent invention of rice fortification is the discovery of a new Ferric pyrophosphate iron absorption (FPP) enhancer (Rohner *et al*., 2017) [40, 41]. In rice extrusion, when trisodium citrate and citric acid were added with isotopically labelled FPP, the absorption of iron from FPP in humans almost doubled to a degree close to that of ferrous sulphate, and the hot extrusion process suggested converting the insoluble FPP to more soluble FPP citrate complexes. No colour changes were registered (Ganz., 2018) [42].

3.1.3 Milk products

To enforce milk with micronutrients, no special equipment is required. The fat-soluble vitamins are initially homogenized with an aliquot of milk, while the water-soluble vitamins and minerals are directly added by hand or by measuring (Hurrell., 2018) [35, 36]. Until packaging, the fortified milk is then agitated, pasteurized, homogenized and heat-treated. Either before or after spray drying, dried milk can be strengthened. Reconstituted dried milk can provide iron for young children as a useful fortifying vehicle (Keats *et al*., 2019) [44]. But because of its relatively high concentrations of calcium and casein, it is a moderate inhibitor of iron absorption. Ascorbic acid is standard practice and can resolve milk-based inhibition and increase iron absorption and effectiveness in young children with the addition of acid to commercial, powdered milk formulas or dried cow's milk fortified with ferrous sulfate (Darton., 2018) [45]. However, the combination of soluble iron compounds with ascorbic acid results in undesirable fluid milk taste changes; the iron strengthening of liquid milk was therefore not widely implemented (Glinz *et al*., 2017) [46].

4. Ferritin

Ferritin was first discovered by Laufberger in the year 1937. Ferritin, the primary iron storage protein was isolated from a horse spleen. The mammal, plant and microbial kingdoms contain ferritins (Zeth *et al*., 2016) [50]. Ferritin is an iron storage protein that consists of a spherical polypeptide shell (Apoferritin) which encloses a 6-nanometer core of the inorganic ferrihydrite hydrochloride (5Fe2O3·9H2O), a variety of amino acid sequences, but the similarity in architecture, are possessed by various ferritins. Spherical proteins consist of 24 subunits of mass 450, 500 kDa and a diameter of about 12 nm and an inner cavity of about 8 nm are part of the ferritin superfamily (Vannucci *et al*., 2015). In contrast to most other high-temperature and pH-sensitive proteins, ferritins can withstand high temperatures up to 75 ºC for ten min and are stable with different denaturants including urea, sodium hydroxide, and guanidinium chloride. The fact that ferritin contains many salt bridges and hydrogen connections forming between subunits makes these unique features possible (Steinman., 2017). Nanoparticles (NPS) are a wide class of materials containing at least one dimension of particulate matter less than 100 nm (Gemede *et al*., 2018). NPS are not simple molecules alone and therefore consist of three layers, i.e. (a) the surface layer that can operate with various molecules, metal ions, surfactants, and polymers. (b) Shell Layer that, in all respects, is chemically different from the core material and (c) the core is basically the central part of the NP and refers generally to the NP itself (Yang *et al*., 2018). Also, recently ferritin has been used to produce nanoparticles for electronics with conduction and magnetic properties based on magnesium, cobalt, and copper (Wang *et al*., 2018). But the regulation of sizes and mineral loads is becoming increasingly clear to manufacture nanomaterials like memory devices and for fluorescent labelling of biomolecules (Chen *et al*., 2018).

Ferritin is an intracellular universal protein that keeps iron and releases it under control. Almost every living organism, including archaea, bacteria, algae, higher plants and animal is responsible for the production of protein. It works as an iron defect buffer and an iron overload in humans (Yang *et al*., 2018). Ferritin is present as cytosolic protein in most tissues, but in limited quantities, it is released into the serum, where it works as an iron carrier. Plasma ferritin also indirectly represents the total amount of iron contained in the body and is also used as an iron deficiency anaemia diagnostic test (Pandolfi *et al*., 2019). It consists of 24 protéin subunits forming nanocages with numerous metal-protein interactions. Ferritin is a globular protein complex. In procaryotic and eucaryotic it is the main intracellular iron-storage protein and maintains iron in a soluble and non-toxic way. Ferritin not mixed with iron is referred to as apoferritin (Puligundla *et al*., 2017).

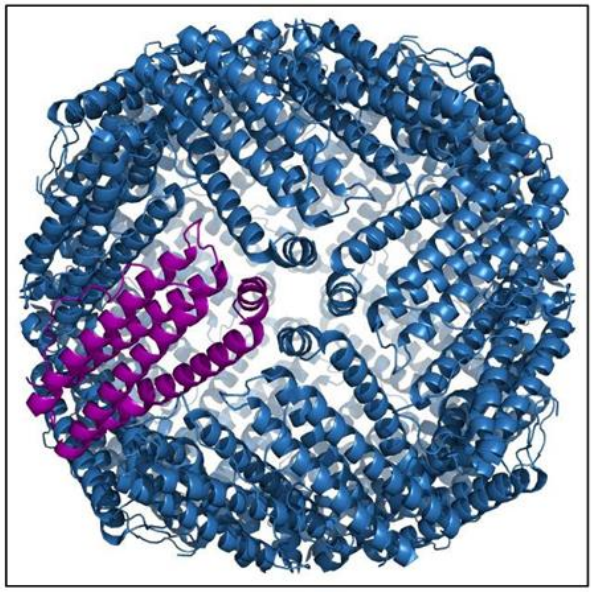

Fig 3: Structure of ferritin core

4.1 Ferritin as a source of iron

In the first place, ferritin-rich foods can only be used as a source of iron, irrespective of the shape they take to the place where they are absorbed (i.e., to the small intestine). But if undamaged ferritin can be passed into the intestine, other chances will arise (Fernandez *et al*., 2018). Endocytosis of ferritin by enterocytes can be a huge opportunity for people who have an increased need for iron intake (e.g., because of blood loss). In the treatment of infectious diseases of the intestine, this mechanism is also promising. Because of its minimal interaction with the atmosphere, ferritin iron is safer. The iron in the protein shell does not touch the food ingredients and/or digestive tract cells. The food should be safe from oxidation or sensory alteration (Hosny., 2015). In addition, oxidative damage can also be limited to intestine cells. The slow release of iron from the ferritin protects the cells of other iron supplements against oxidative damage. There are other difficulties with the production of bioactive food with a high ferritin iron content. The concentration of ferritin iron in food of plant origin varies with the botany of the seeds or grains used. However, it also results from crop cultivation conditions (especially soil iron concentration) and food processing (Izquierdo., 2016). Since ferritin is a protein, food should be processed with non-thermal denaturation conditions. Therefore, the temperature or the exposure period should be restricted to elevated temperatures. The method of iron speciation in plant samples was shown to have survived some thermal treatment, although the temperatures were above those which were known to degrade protein, i.e. more than 80 °C. Samples spike with labelling ferritin bean and mass ionisation of thermal ionisation spectrometry isotope dilution (IDMS) enables ferritin iron quantification. The colourimetric analysis of the various iron shapes taken from food samples, organically and inorganically complex as well as the ionic form of iron are another approach that is worth considering (Zhang *et al*., 2015).

4.2 Ferritin nanoparticle

The use of NPS was proposed in iron supplementation and enforcement as their use could include high bioavailability, good product stability, minimal adverse effects and lack of changes in flavour and colour fortified foodstuffs (Gozzelino *et al*., 2016). In addition, experiments *in vitro* and *in vivo* revealed that iron NPs are healthy. The fortification of animal foods also included nanoformulations. Well-absorbed ferritin is made of an iron oxide nanocore and has been recently presented as a possible side-effect-free source of inexpensive, supplementary iron. The nanoparticulate mimetic of the ferritin core is also presented (Cappellini *et al*., 2015). Iron nanoparticulate can follow another pathway to enterocyte cytoplasm. Endocytosis is the canonical mechanism used by NPs, but it may not be the only one (Izquierdo *et al*., 2016).

When applied to the polymer matrix, nanoparticles can quickly be aggregated and agglomerated. During the manufacturing, process nanoparticles may be aggregated and/or incorporated into polymers (Zappalorto *et al*., 2015). Aggregation, as well as agglomeration, are clusters of nanoparticles with aggregations referred to as solid and compact particle collectives. Agglomeration also occurs when particles are loosely combined and can be broken down by mechanical forces (Jin *et al*., 2015). The agglomeration prevents improved properties from being achieved and, thus, various applications are achieved with nanostructured materials. The agglomeration is a direct reciprocal attraction between nanoparticle forces or chemical links through the van der Waals. The methods to prevent aggregation are primarily due to capping, coupling or compatibility of the particles and loading the surface of the filler to isolate the filler using an electrostatic spillage (Fasihi *et al*., 2016). Furthermore, optimal output parameters will result in a successful aggregate breakdown. For example, in extruder melting, an ideal velocity and feeding rate are highly stressful for melting materials that disturb the aggregates of the particles (Zare *et al*., 2017).

5. Ferritin nanoparticles structure

Structure Ferritin nanoparticles ubiquitously found in nature contain a hollow core of inner and outer diameters of 8 and 12 nm, respectively, that can internalize up to 4500 iron atoms in the form of ferric oxyhydroxide, with variable amounts of phosphate (Zeth *et al*., 2016) [50]. The ferritin structure of mammalians, amphibians, plants, and bacteria has been reviewed elsewhere (Zhen *et al*., 2013). Although there are considerable variations in the amino acid sequence (up to 80%) and the presence of haem moieties in some bacterial ferritins, their tertiary structure is quite similar and some subdomains such as the iron entry/exit channels contain highly conserved sequences across different species (Tian *et al*., 2016). Ferritin particles isolated from vertebrates are composed of two types of subunits, H-chain (heavy, 21 kDa) and L-chain (light, 19 kDa), whereas those found in plants and bacteria contain only one type resembling the H- chain of vertebrates (Kitagawa *et al*., 2012). Each ferritin particle is made up of 24 identical or homologous subunits that self-assemble in octahedral (432) symmetry such that small channels are formed at the 4-fold and 3-fold symmetry axes. These channels allow the passage of iron and other ions or small molecules, with iron being guided via the 3-fold channels (Falvo *et al*., 2013). Each monomer is composed of a four-α-helix bundle (A, B, C, and D helices) with a short fifth E-α-helix at the C-terminus that runs from the outside to the inside, making the C- terminal end of the ferritin chain to be inside the assembled nanoparticle. Additionally, a long loop (L) of about nineteen residues connects the C-terminal of

the B-helix to the N-terminal of the C-helix. The N-terminal, L loop, and A- and C-helices are solvent-accessible, while the C- terminal, and B- and D-helices face the inner side of the ferritin nanocage (Mamo and Poland., 2012).

5.1 Stabilization of iron nanoparticles

In many manufacturing technologies and industrial goods, stabilization of nanoparticles in condensed aqueous suspensions is essential. The adsorption of a dispersant coating across the surface of the particles typically stabilizes nanoparticles. For the stabilization of suspensions containing high nanoparticle concentrations, the formation of the required thickness dispersants (adlayer) is essential (Purcar *et al*., 2017). Thick adlayers contribute to excessive excluded volume, while thin adlayers cause agglomeration of the particles. The maximum suspension nanoparticle concentration is reduced in both effects however, traditional dispersants cannot systematically track the thickness of the adlayer on the surface of the particles (Caprarescu *et al*., 2015). In many current and future technologies for device manufacturing, suspensions with high contents of nanoparticles (>40 vol%) may be very beneficial, if not the main requirement. Several changes have been made to the surface and stabilization methods utilizing different kinds of add-ons such as surfactants, polymers, water- soluble starch, carboxymethyl cellulose, cellulose acetate, polyacrylic acid, etc to resolve these constraints (Ahmad *et al*., 2015). These additives effectively regulate the form and size of nanoparticles, improve the stability and mobility of nanoparticles, and thus improve the efficiency of nanoparticles (Podporska *et al*., 2017).

6. Characterization

6.1 Zeta potential

Dynamic light dispersion is the technique used to determine the size distribution profile for silver nanoparticles dispersed in deionized water in material physics. The size distribution profile of the nanoparticles in the final solution is calculated after ultracentrifuge using light dispersion techniques. Research has found that the average NPS particle size is hydrodynamic (Lowry *et al*., 2015).

6.2 Uv-visible spectroscopy

UV-Vis spectroscopy in the spectral area of ultra-violet and visible spectra can be understood as absorption spectroscopy. It usually uses visible and almost UV light. Ultraviolet and visible light is powerful enough to increase external electrons and the spectroscopy of UV-Vis is normally applied to solvent molecules (Cahyana *et al*., 2017). The UV-Vis spectrum has broad functions, which are restricted in sample identification but are very helpful for quantitative measurements. By measuring absorption at a particular wavelength and by applying the BeerLambert Act the concentration of analytes in the solution can be calculated (Gemede *et al*., 2018). As the UV-Vis range covers approximately 400-750 nm of human visual acuity, UV-Vis spectroscopy serves as a characteristic for the absorption, transmission and reflection of a range of technologically important materials, such as pigments, coatings etc. The mucilage nanoparticles will be scattered in a 2 mg/ml dilute suspension using a 30-minute bath sonicator into MilliQ water. The absorption strength of the particles will be calculated using the visible UV spectrophotometer when fully dispersed in water (Zhang *et al*., 2016).

6.3 Differential scanning colourimetry

To investigate the heat-effectiveness of the sample under argon atmosphere and DSC analysis at a rate of 10 °C/min under argon atmosphere from 25 °C and a differential scanning calorimetry (DSC 302 BUHR, Germany) will be performed at a heating speed of 25 °C/min (Zhang *et al*., 2016).

7. Food fortification and application

Fortification is defined as the addition of one or more essential elements to a food article, whether it has previously been added to the food, to prevent or correct nutrient deficiency in the general population or a specific population group. Food fortification with minerals and vitamins aids in the eradication of diseases like goitre, rickets, beriberi, and pellagra. Significant progress has also been made in the prevention of anaemia and vitamin A deficiency (Omoruyi *et al*., 2013). The mentioned deficiencies can be prevented and eliminated through appropriate and diverse nutrition and supplementation of deficient micronutrients, but food fortification is the best solution on a national scale. The application of fortification is predicated on two basic conditions: that the food item is widely consumed and that it is inexpensive (available) (Sparvoli and Cominelli., 2015). The goal of our paper was to show the results of fortification in various countries to lay the groundwork for similar propositions in our country (Serbia and Montenegro). The number of cretinism cases in Asia has been cut in half because of fortification, while sugar fortification has significantly reduced the number of children with vitamin A deficiency (Yao *et al*., 2015). For more than 50 years, flour fortification with iron to prevent iron deficiency and anaemia has been used successfully in the United States and Canada, and more recently in some African and South American countries. The results indicate that food fortification has had a positive impact on health in the communities where it has been implemented (Ani and Abel., 2018).

8. Conclusion

Although iron supplementation has a greater impact on health than fortification, iron fortification is less expensive and has the major advantage of aligning with the human body's functioning and physiology. As a result, fortification remains the most secure intervention to address the issue of iron deficiency. For long-term applicability, various technical opportunities, such as the safety and efficacy of nanotechnology in food fortification, must be investigated. There is a need for a better understanding of nutrient bioavailability as well as solutions to organoleptic and degradation issues associated with fortifiers. Most efforts to combat iron deficiency through fortification strategies have thus far focused on overcoming technical issues such as discolouration, off-flavour development, and fatty acid oxidation, often overlooking other practical issues such as large-scale production, marketing, and quality control, which are critical for successful implementation. There is still a need for novel food carrier systems that allow for correlation with food processing techniques. To do so, a better understanding of dietary components, processing methods, and physiological factors is required. This must then be linked to the chemodynamics of iron during food processing and after

ingestion, as this determines the mineral's fate during processing and digestion. Individual applications must also address issues such as iron compound stability, powder/product characteristics, toxicity, and sustainability.

9. References

1. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loréal O. Haemochromatosis. Nat Rev Dis Primers, 18016, 2018.
2. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta. 2012;1823(9):1434-1443.
3. Drakesmith H, Nemeth E, Ganz T. Ironing out Ferroportin. Cell Metab. 2015;22(5):777-787
4. Ramakrishnan SK, Anderson ER, Martin A, Centofanti B, Shah YM. Maternal intestinal HIF-2α is necessary for sensing iron demands of lactation in mice. Proc Natl Acad Sci U S A. 2015;112(28):E3738–E3747.
5. Anderson ER, *et al*. Intestinal HIF2α promotes tissue-iron accumulation in disorders of iron overload with anemia. Proc Natl Acad Sci U S A. 2013;110(50):E4922-E4930.
6. Berdoukas V, Coates TD, Cabantchik ZI. Iron and oxidative stress in cardiomyopathy in thalassemia. Free Radic Biol Med. 2015;88(Pt A):3-9.
7. Triner D, Xue X, Schwartz AJ, Jung I, Colacino JA, Shah YM. Epithelial hypoxia-inducible factor 2α facilitates the progression of colon tumors through recruiting neutrophils. Mol Cell Biol. 2017;37(5):e00481-16.
8. Xue X, Ramakrishnan SK, Shah YM. Activation of HIF-1α does not increase intestinal tumorigenesis. Am J Physiol Gastrointest Liver Physiol. 2014;307(2):G187-G195.
9. Qiao B, *et al*. Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. Cell Metab. 2012;15(6):918-924.
10. Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature. 2014;509(7498):105-109.
11. Ross SL, *et al*. Molecular mechanism of hepcidin-mediated ferroportin internalization requires ferroportin lysines, not tyrosines or JAK-STAT. Cell Metab. 2012;15(6):905-917.
12. Lakhal-Littleton S, *et al*. Cardiac ferroportin regulates cellular iron homeostasis and is important for cardiac function. Proc Natl Acad Sci U S A. 2015;112(10):3164-3169.
13. Evstatiev R, Gasche C. Iron sensing and signalling. Gut. 2012;61(6):933-52.
14. Hudak L, Jaraisy A, Haj S, *et al*. An updated systematic review and meta-analysis on the association between Helicobacter pylori infection and iron deficiency anemia. Helicobacter, 2016.
15. Hershko C, Camaschella C. How I treat unexplained refractory iron deficiency anemia. Blood, 2014
16. Vaucher P, Druais PL, Waldvogel S, *et al.* Effect of iron supplementation on fatigue in nonanemic menstruating women with low ferritin: a randomized controlled trial. CMAJ, 2012.
17. Sharma R, Stanek JR, Koch TL, *et al.* Intravenous iron therapy in non-anemic irondeficient menstruating adolescent females with fatigue. Am J Hematol, 2016.
18. Cohen-Solal A, Leclercq C, Deray G, *et al.* Iron deficiency: an emerging therapeutic target in heart failure. Heart, 2014
19. Von HS, Jankowska EA, van Veldhuisen DJ, *et al*. Iron deficiency and cardiovascular disease. Nat Rev Cardiol, 2015
20. Qian C, Wei B, Ding J, *et al*. The efficacy and safety of iron supplementation in patients with heart failure and iron deficiency: A systematic review and meta-analysis. Can J Cardiol, 2016.
21. Robinson JC, Graham BB, Rouault TC, *et al*. The crossroads of iron with hypoxia and cellular metabolism. Implications in the pathobiology of pulmonary hypertension. Am J Respir Cell Mol Biol, 2014.
22. Evstatiev R, Gasche C. Iron sensing and signalling. Gut. 2012;61(6):933–52.
23. Levi M, Rosselli M, Simonetti M, *et al*. Epidemiology of iron deficiency anaemia in four European countries: a population-based study in primary care. Eur J Haematol, 2016.
24. Pantopoulos K, Porwal SK, Tartakoff A, *et al*. Mechanisms of mammalian iron homeostasis. Biochemistry 2012;51(29):5705–24
25. Pavord S, Myers B, Robinson S, *et al*. UK guidelines on the management of iron deficiency in pregnancy. Br J Haematol. 2012;156(5):588–600.
26. Hudak L, Jaraisy A, Haj S, *et al*. An updated systematic review and meta-analysis on the association between Helicobacter pylori infection and iron deficiency anemia. Helicobacter, 2016.
27. Hershko C, Camaschella C. How I treat unexplained refractory iron deficiency anemia. Blood. 2014;123(3):326–33.
28. Weng TC, Chang CH, Dong YH, *et al*. Anaemia and related nutrient deficiencies after Roux-en-Y gastric bypass surgery: A systematic review and meta-analysis. BMJ Open. 2015;5(7):e006964.
29. Ruz M, Carrasco F, Rojas P, *et al*. Heme- and nonheme-iron absorption and iron status 12 mo after sleeve gastrectomy and Roux-en-Y gastric bypass in morbidly obese women. Am J Clin Nutr. 2012;96(4):810-7.
30. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: a systemic review. Asian Pac. J Trop. Biomed. 2015;5:601-611.
31. Chandrasekara A, Daugelaite J, Shahidi F. DNA scission and LDL cholesterol oxidation inhibition and antioxidant activities of Bael (*Aegle marmelos*) flower extracts. J Tradit Complement Med. 2018;8:428-435.
32. Bacanlı M, Başaran AA, Başaran N. The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin. Food Chem. Toxicol 2015;81:160-170.
33. Ansari P, Afroz N, Jalil S, Azad SB, Mustakim MG, Anwar S, *et al*. Anti-hyperglycemic activity of *Aegle marmelos* (L.) corr. is partly mediated by increased insulin secretion, α-amylase inhibition, and retardation of glucose absorption. J Pediatr. Endocrinol. Metab. 2017;30:37-47.
34. Bamzai K, Bhat M. Electrical and magnetic properties of some rare earth orthoferrites (RFeO3 where R ¼ Y, Ho, Er) systems, Integr. Ferroelectr, 2015, 158.
35. Zimmerman SL, Montgomery S. Grain fortification processes, technologies, and implementation criteria. In: Venkatesh Mannar MG, Hurrell RF, editors. Food fortification in a globalized world. London: Academic

Press, 2018, 85-92.
36. De Pee S, Tsang BL, Montgomery S. Rice fortification. In: Venkatesh Mannar MG, Hurrell RF, editors. Food fortification in a globalized world. London: Academic Press, 2018, 131-41.
37. Hackl L, Cercamondi CI, Zeder C, Wild D, Adelmann H, Zimmermann MB, *et al*. Cofortification of ferric pyrophosphate and citric acid/trisodium citrate into extruded rice grains doubles iron bioavailability through in situ generations of soluble ferric pyrophosphate citrate complexes. Am J Clin Nutr. 2016;103:1252-9
38. Diosady LL, Mannar MGV, Krishnaswamy K. Improving the lives of millions through the new double fortification of salt technology. Matern Child Nutr. 2019;15:12773.
39. Cercamondi CI, Duchateau GSMJE, Harika RK, Van, Den, Berg R, Murray P, *et al*. (Sodium pyrophosphate enhances iron bioavailability from bouillon cubes fortified with ferric pyrophosphate. Br J Nutr. 2016;116:496-503.
40. Namaste SM, Rohner F, Huang J, Bhushan NL, Flores-Ayala R, Kupka R, *et al*. Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106:359S-71S.
41. Rohner F, Namaste SM, Larson LM, Addo O, Mei Z, Suchdev PS, *et al*. Adjusting soluble transferrin receptor concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr. 2017;106:372S-82S
42. Ganz T. Iron and infection. Int J Hematol. 2018;107:7-15.
43. Hurrell RF. Efficacy and safety of iron fortification. In: Venkatesh Mannar MG, Hurrell RF, editors. Food fortification in a globalized world. London. Academic Press, 2018, 196-212.
44. Keats EC, Neufeld LM, Garrett GS, Mbuya MNN, Bhutta ZA. Improved micronutrient status and health outcomes in low- and middle-income countries following large-scale fortification: evidence from a systematic review and meta-analysis. Am J Clin Nutr. 2019;109:1696-708.
45. Darton-Hill I. Prevalence, causes and consequences of micronutrient deficiencies. In: Venkatesh Mannar MG, Hurrell RF, editors. Food fortification in a globalized world. London: Academic Press, 2018, 13-28.
46. Glinz D, Wegmüller R, Ouattara M, Diakité V, Aaron G, Hofer L, *et al*. Iron-fortified complementary foods containing a mixture of sodium iron EDTA with either ferrous fumarate or ferric pyrophosphate reduce iron deficiency anaemia in 12- to 36-monthold children in a malaria- endemic setting: a secondary analysis of a cluster-randomized controlled trial. Nutrients. 2017;9:759.
47. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, *et al*. Biomarkers of Nutrition for Development (BOND)—an iron review. J Nutr. 2018;148:1001S-67S.
48. Jung J, Rahman Md. S, Rahman Md. S, Swe KT, Islam MdR, Rahman Md. O, *et al*. Effects of haemoglobin levels during pregnancy on adverse maternal and infant outcomes: a systematic review and meta-analysis. Ann N Y Acad Sci. 2019;1450:69-82.
49. Klassen-Wigger P, Geraets M, Messier MC, Lenoble HP, Barclay DV. Micronutrient fortification of bouillon cubes in West Africa. In: Venkatesh Mannar MG, Hurrell RF, editors. Food fortification in a globalized world. London: Academic Press, 2018, 363-72.
50. Zeth K, Hoiczyk E, Okuda M. Ferroxidase-mediated iron oxide biomineralization: Novel pathways to multifunctional nanoparticles. Trends in Biochemical Sciences. 2016;41:190-203.