CHAPTER 1

INTRODUCTION

Vitamins and different elements/minerals are essential for the human body to perform various biological, chemical, and molecular functions. The deficiency of these vitamins and minerals in the body leads to malnutrition. The most common symptoms of malnutrition are fatigue, dizziness, and weight loss. Prolonged deficiency without any treatment leads to mental and physical disability. It is found that 98% of human body mass is constituted with nine non-metallic elements in which sodium, calcium, potassium, and magnesium account for 1.89% [1].

Different important trace elements that are in need to the body include zinc (Zn), iron (Fe), selenium (Se), copper (Cu), cobalt (Co), chromium (Cr), manganese (Mn), iodine (I), and molybdenum (Mo). Even though these metals constitute only 0.02% of the body, they play a significant role in carrying the various metabolic and physiologic processes of the organism throughout life [2]. The inadequate occurrence of these trace metals in diets of humans leads to trace metal malnutrition or hidden hunger. Since each a trace element is associated with many enzyme reactions, the deficiency due to deficiency of a trace element will be a combination of different clinical manifestations. For example, Zn, Cu, and Mn are associated with the function of bone metabolism. The physiological function of carbohydrate metabolism occurs in the presence of Zn, Cr, and Mn [2]. Due to rapid urbanization and economic development preferences towards nutritionally deprived junk food and refined diets results in poor nutrition leading to impaired physical and mental growth, and reduced immunity [3]. People struggling with poverty were found to be more vulnerable to hidden hunger due to a variety of reasons like the existence of scanty resources with little finances for access to healthy foods, stress, cycle of food deprivation and overeating, less opportunity for physical activity, and scanty access to health care along with added resource-related hardships like energy insecurity and housing instability which likely leads to poor health and nutrition [4, 5].

Among different trace elements, iron is one of the essential and abundant metals that is present in humans. A total content of 3 – 5 g of Fe was found to be present with the maximum amount in the blood, and the remaining in liver, bone marrow, and muscles [3]. Heme, which is the major form of Fe, exists in cytochrome, myoglobin, and hemoglobin. Iron is also linked with various enzymes like oxidases, peroxidases, and dehydrogenases involved in various metabolic reactions for the generation of energy. The major oxygen-carrying pigment of mammals is hemoglobin. Hence, iron plays a vital role in carrying oxygen to various tissues. Besides its physiological role, iron is also necessary for the synthesis of DNA (Deoxyribonucleic acid), RNA (Ribonucleic acid), antibody, and collagen [6]. So the role of iron in the human body is considered as significant in comparison with the other trace metals.

Iron depletion in the body leads to iron deficiency. Since most of the iron is utilized in the formation of hemoglobin, anemia is considered a noticeable sign of iron deficiency. It leads to impairment of cognitive performance in young children and decline in elders/ reduction of physical and working capacities in adults, and adverse pregnancy outcomes for both newborns and mothers [7]. Infants at the time of their growth, preschool children, young menstruating women, and women during pregnancy and postpartum, were found to be most vulnerable for iron deficiency anemia [8]. Global Burden of disease 2016, reveled that anemia caused by iron deficiency is one of the five leading causes for living with disability burden [9]. In 2010, a worldwide survey showed that one-third of the population is still affected with anemia, of which 50% of cases are due to iron deficiency. It is estimated that 1.24 billion individuals experienced iron deficiency anemia [10]. Around 1.7 billion people worldwide are affected with iron deficiency anemia with a prevalence of 25.1% globally [11]. Among the developing countries, the anemia prevalence was found to be more in India, and it is also one of the (second) leading causes for maternal deaths [12]. As per National Family Health Survey (NFHS) 2016, anemia prevalence was found to be more in children (58.6%) followed by non-pregnant (53.2%) and pregnant women (50.4%) [13].

Iron deficiency in most of the individuals is nutritional, which is due to inadequate intake of foods rich in bioavailable iron, reduced capacity of absorption, and gastrointestinal blood losses [7]. According to the World Health Organization, anemia is defined by levels lower than 12 g/dl in non-pregnant women, 13 g/dl in men, and 11 g/dl in pregnant women. Hence, care must be taken before reaching the borderline values [14]. Considering the demand for all age groups, oral iron supplementation was found to be effective, inexpensive, and convenient. However, its application is limited only to mild anemia since there will be slow repletion. When repletion was desired to occur faster, the preferred route of administration is intravenous which is expensive and requires supervision by professional health care personnel [15]. By considering associated disadvantages into account, fortification of food with iron was found to be an interesting, productive strategy for addressing the prevalence of iron-deficient anemia. Supplementation of diet with iron-fortified food will be a sustainable solution to acquire an adequate amount of bioavailable iron.

The majority of the market available fortification technologies rely on the application of inorganic salts of iron. However, iron when present in food interacts with the food components and causes metallic taste, an impermissible flavour which is due to lipid/fat oxidative rancidity, disagreeable colour changes by interacting with anthocyanin's, flavonoids, tannins, and vitamin A as well as Vitamin C (essential for the absorption of iron) and mineral degradation (i.e., iodide/iodate were oxidized to free iodine and escapes as a gas) leads to lower consumer acceptance [16]. Organic iron complexed/chelated with ligands like proteins, amino acids and polysaccharides displayed a better bioavailability. Since iron in organic form is complexed with ligands, it may not react with other food components and hence the associated oxidative nutritional deterioration can be arrested making the final food organoleptically acceptable [16-29].

Microorganisms are known to possess metal binding capacity due to the occurrence of various functional groups on their surface [30]. Biosorption is a surface phenomenon where the metal ion of interest will bind to the biological matrix either by chemical (proton or ion displacement)/physical (electrostatic forces) interactions or by chelation or complexation mechanisms. Whereas bioaccumulation/uptake is a metabolically active process which involves the uptake of metal ions into the intercellular space of the microorganisms by using importer complexes. The metals thus can be sequestered into the cell by peptide or protein ligands [31]. Many microorganisms have been characterized for their metal binding capacity either by using biosorption [32-36] or uptake mechanism [37-39].

On the other side it is also crucial to evaluate the bioaccessibility and bioavailability of the fortified nutrient upon ingestion. The conventional methods used for the determination of nutrient bioavailability can be classified into *in vitro* and *in vivo* techniques.

The *in vitro* simulated gastrointestinal digestion of food involves the mimicking of digestion that occur in the human digestive tract with the addition of respective digestive enzymes such as pepsin, bile salts and pancreatin to determine the bioaccessibility of nutrients by simulating the pH. Many research studies determined the bioaccessibility of different metal ions from various food sources like, sea foods, vegetables and rice by this method [40-42]. The efficacy and effectiveness of a product to improve the nutritional status can be evaluated by conducting *in vivo* (animal) experiments. Information regarding the bioavailability of ingested nutrient can be obtained by using different animal models by maintaining at suitable environmental conditions. The bioavailability of nutrient (metal) from different fortified food products were evaluated in many studies using rats or mice as the experimental models [43-45].

It is important to select the food matrix that is acceptable to the population of all age groups. Fermented milk (Dahi) is typical of all dairy products and it occupies prominent place in the daily diet of most of the population. Chocolate will be an attractive solution for addressing iron deficiency in children [46] because they constitute about half of the population affecting by iron deficiency anemia. Also, as children are fond of chocolate-flavored foods, it can be a suitable vehicle.

Hence, in the present study, three food-grade microorganisms namely, *Lactobacillus fermentum*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* were characterized for biosorption of iron from aqueous solutions. The bioavailability of iron-bound microbial biomass was evaluated by simulated gastrointestinal (*in vitro)* model and animal (*in vivo)* model. The obtained iron-bound microbial biomass was used for fortification of curd/dahi and chocolate and inhibition of oxidative changes was studied.