# 2

# Review of Literature

The review of literature pertaining to the present study on **"Development and Evaluation of *Ulva lactuca* based Probiotic Beverage and *in vitro* Bioavailability of Iron using Caco-2 Cell Model"** is discussed under the following headings:

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## 2.1. Global statistics adverting to Iron as an essential micronutrient and Iron Deficiency Anemia (IDA).

Iron is an essential micronutrient that plays a crucial role in various physiological processes, including oxygen transport, DNA synthesis, and energy metabolism. Globally, iron deficiency is the most prevalent nutritional disorder, affecting billions of people and leading to Iron Deficiency Anaemia (IDA), particularly among vulnerable populations such as children, women of reproductive age, pregnant and lactating women. The World Health Organization (WHO) estimates that approximately 30% of the world's population is anaemic, with iron deficiency being the leading cause in 50% of cases (WHO, 2021).

Globally, the prevalence of IDA varies widely, influenced by dietary patterns, socioeconomic status and healthcare access. In low- and middle-income countries (LMICs), poor dietary intake, high infection rates, and limited health services exacerbate the prevalence of iron deficiency (Pasricha *et al.,* 2013). In high-income countries, specific populations, such as pregnant women and young children, are still at risk due to increased iron requirements and suboptimal dietary intake (McLean *et al.,* 2009).

In India, the prevalence of iron deficiency and IDA is alarmingly high. The National Family Health Survey (NFHS-5) reported that 53% of women aged 15-49 and 58.4% of children aged 6-59 months were anaemic (IIPS, 2016). These figures underscore the public health challenge posed by iron deficiency in the country. Despite numerous interventions, including iron supplementation and food fortification programs, the burden of IDA remains substantial.

The physiological necessity of iron stems from its role as a component of haemoglobin, the molecule responsible for oxygen transport in the blood. Iron is also a critical element of myoglobin in muscles and various enzymes involved in cellular respiration and DNA synthesis (Beard *et al.,* 2001). Consequently, insufficient iron levels impair oxygen delivery to tissues, leading to fatigue, weakened immunity, and impaired cognitive and physical performance (Zimmermann *et al.,* 2007).

The consequences of iron deficiency and IDA extend beyond health, impacting economic productivity and national development. Anaemia in adults can reduce work capacity by up to 20%, translating into substantial economic losses, particularly in LMICs where physical labour is a major component of the workforce (Horton *et al.,* 2003). For children, iron deficiency can impair cognitive development and educational attainment, perpetuating cycles of poverty and malnutrition (Lozoff *et al.,* 2006). Narsinga Rao *et al.,* (2007) have reported that serum ferritin was significantly correlated with intake of niacin, vitamin B12, and selenium.

### 2.1.1. Iron uptake and transport pathway in healthy individuals and IDA – an overview.

Iron uptake and transport are critical physiological processes, essential for maintaining cellular function and overall health. In healthy individuals, dietary iron is absorbed primarily in the duodenum and upper jejunum, where it exists in two forms: heme and non-heme iron. Heme iron, derived from haemoglobin and myoglobin in animal products, is efficiently absorbed via the heme carrier protein 1 (HCP1) (Anderson *et al.,* 2016). Non-heme iron, predominantly from plant sources, undergoes reduction from Fe3+ to Fe2+ by duodenal cytochrome b (Dcytb) before being transported into enterocytes by divalent metal transporter 1 (DMT1) (Smith *et al.,* 2017).

Once inside enterocytes, iron is either stored as ferritin or transported across the basolateral membrane by ferroportin (FPN), aided by the ferroxidase activity of hephaestin (Chen et al., 2018). The systemic regulation of iron homeostasis is primarily governed by hepcidin, a liver-produced hormone that inhibits FPN, thereby reducing iron efflux from enterocytes and macrophages (Ganz *et al.,* 2017). Hepcidin expression is modulated by various factors, including iron status, erythropoietic activity, and inflammatory signals (Meynard *et al.,* 2018).

In individuals with Iron Deficiency Anaemia (IDA), the regulatory mechanisms of iron metabolism are disrupted. Lower hepcidin levels are observed, leading to increased FPN activity and iron absorption (Nemeth *et al.,* 2017). Despite this compensatory increase, iron absorption often remains inadequate due to underlying causes such as chronic blood loss, malabsorption syndromes, or increased physiological demands (Pietrangelo, 2017). The upregulation of DMT1 and Dcytb further highlights the body's attempt to enhance iron uptake during deficiency (Santos *et al.,* 2019).

Recent studies have also identified novel genetic mutations affecting iron transport proteins, which contribute to the variability in IDA manifestations and treatment responses (Steinbicker *et al.,* 2018). Furthermore, therapeutic strategies targeting hepcidin modulation are under investigation to improve iron availability in IDA patients (Kroot *et al.,* 2019). Understanding these intricate pathways and regulatory mechanisms is crucial for developing effective treatments for iron-related disorders.

Hepcidin, a key regulator of iron homeostasis, has emerged as a significant biomarker for Iron Deficiency Anaemia (IDA) in recent research. Several studies have consistently highlighted its diagnostic and prognostic utility. For instance, Ganz *et al.,* (2017) established that serum hepcidin levels are markedly reduced in IDA, reflecting decreased iron stores and enhanced iron absorption. Similarly, research by Girelli *et al.,* (2016) demonstrated the inverse correlation between hepcidin levels and transferrin saturation, underscoring its role in iron metabolism assessment.

Further studies have expanded on these findings. The work of Arezes *et al.,* (2018) confirmed that hepcidin levels could distinguish between IDA and anaemia of chronic disease (ACD), a differentiation critical for appropriate treatment strategies. Moreover, Pagani *et al.,* (2019) illustrated that hepcidin measurements are not only reliable but also superior to traditional markers like ferritin and transferrin receptors in diagnosing IDA, particularly in complex clinical scenarios involving inflammation or chronic conditions.

Kroot *et al.,* (2020) emphasized the dynamic nature of hepcidin, noting that its levels fluctuate in response to iron therapy, providing real-time insights into treatment efficacy. This real-time monitoring capability was further corroborated by Nemeth *et al.,* (2021), who showed that hepcidin levels rise predictably with intravenous iron administration, suggesting its potential use in monitoring therapeutic outcomes.

In pediatric populations, studies by De Falco *et al.,* (2018) and McCarthy *et al.,* (2020) have demonstrated that hepcidin is a valuable marker for the early detection of IDA, facilitating timely interventions. Additionally, economic evaluations by Schwartz *et al.,* (2019) have highlighted the cost-effectiveness of incorporating hepcidin assays into routine diagnostic protocols, given their high sensitivity and specificity.

Collectively, these studies underscore the robustness of hepcidin as a biomarker for IDA, advocating for its broader implementation in clinical practice to enhance diagnostic accuracy and treatment efficacy.

### 2.1.2. Sources of food and edible underexploited seaweeds as potential sources of Iron.

Addressing iron deficiency and IDA requires a multifaceted approach, combining dietary diversification, supplementation, fortification, and public health education. Enhancing the bioavailability of dietary iron through the inclusion of vitamin C-rich foods and reducing inhibitors of iron absorption can significantly improve iron status (Lynch *et al.,* 2000). Moreover, strengthening health systems to ensure effective delivery and monitoring of iron interventions is crucial (Bhutta *et al.,* 2013).

Oranges and whey have been shown to have both synergistic and inhibitory effects on iron bioavailability in diet, according to recent studies. Ascorbic acid-rich oranges have been repeatedly demonstrated to improve the absorption of non-heme iron. Ascorbic acid from oranges dramatically increased iron bioavailability from plant-based diets by converting ferric iron to the more absorbable ferrous form, according to a study by Ofori-Mensah *et al.,* (2017). Li *et al.,* (2018) also found that eating orange juice with meals improved iron absorption in both omnivorous and vegetarian subjects.

Conversely, whey, a by-product of cheese production, contains lactoferrin and other proteins that may affect iron bioavailability differently. According to a study by Brune *et al.,* (2016), lactoferrin in whey can bind iron, potentially enhancing its bioavailability by protecting it from inhibitors such as phytates and polyphenols. However, findings by Aranda *et al.,* (2019) indicate that whey protein might inhibit iron absorption by forming insoluble complexes at certain pH levels in the gut. This was supported by research from Gonzalez *et al.,* (2020), who found that while whey can increase iron uptake in low-phytate environments, it may reduce absorption in the presence of high-phytate foods.

Research conducted by Zhang *et al.,* (2021), reveals that the combination of oranges and whey produces varying consequences depending on the dietary environment, implying the need for individualized dietary recommendations. Furthermore, research such as Silva *et al.,* (2022) underline the significance of meal composition in influencing the overall influence on iron bioavailability. Overall, our data highlight the intricate interplay between dietary components and the importance of comprehensive dietary regimens to enhance iron absorption.

Seaweeds, particularly underexploited varieties, have gained attention as potential sources of iron due to their nutritional benefits and sustainability. Several studies have explored the iron content in various seaweeds, highlighting their potential to address iron deficiency, a prevalent global nutritional issue. Firstly, research by Mohamed *et al.,* (2016) emphasizes that seaweeds like Gracilaria and Sargassum contain significant levels of iron, making them suitable for dietary inclusion to combat anemia. Similarly, Kumar *et al.,* (2017) identify *Ulva lactuca* as a rich source of iron, noting its potential in nutritional supplements. Furthermore, Kumar *et al.,* (2018) report that *Kappaphycus alvarezii,* another underutilized seaweed, exhibits high iron content, advocating its use in food fortification programs. This is corroborated by Pereira (2018), who identified *Porphyra* species as not only rich in iron but also containing other essential micronutrients, enhancing their nutritional profile. Venkatesan *et al.,* (2019) provide a comprehensive analysis of various seaweeds, including *Eisenia bicyclis* and *Laminaria digitata*, which are identified as substantial sources of iron. A study by Abirami and Kowsalya, 2011 reported *Ulva spp.* to be a good source of Iron. Their study suggests the inclusion of these seaweeds in functional foods to enhance iron intake. In a related study, Dawczynski *et al.,* (2020) highlight the bioavailability of iron in seaweeds, particularly noting that *Palmaria palmata* has iron absorption rates comparable to conventional iron sources. This finding is significant as it underscores the practicality of using seaweeds in addressing iron deficiency. Hamed *et al.,* (2021) examine the potential of *Ascophyllum nodosum* and *Fucus vesiculosus* as iron-rich dietary sources, highlighting their benefits in improving nutritional status without the side effects associated with synthetic iron supplements.

In the context of sustainable nutrition, Sharma *et al.,* (2021) argue for the inclusion of underexploited seaweeds in diet plans, pointing out their high iron content and minimal environmental footprint. They emphasize the need for further research to optimize harvesting and processing techniques to maximize nutritional benefits. Studies by Parthiban *et al.,* (2022) and Karimi *et al.,* (2022) suggest that seaweeds such as *Chondrus crispus* and *Codium fragile* offer significant iron content and should be explored further for their potential in dietary applications. It could be inferred that the exploration of underexploited seaweeds as sources of iron and the use of orange juice and whey for a synergistic effect presents a promising avenue for enhancing dietary iron intake and addressing nutritional deficiencies. The cited studies collectively highlight the substantial iron uptake efficacy, advocating their inclusion in functional foods and nutritional supplements to promote public health.

### 2.1.3. Factors affecting iron bioavailability from food.

In India, dietary patterns heavily reliant on plant-based foods contribute to the high prevalence of iron deficiency. One of the key nutrients enhancing iron absorption is vitamin C, which reduces ferric iron to the more soluble ferrous form, thereby increasing its uptake (Cook *et al.,* 2017). Similarly, animal-based proteins such as meat, fish, and poultry (known as the "meat factor") have been shown to improve non-heme iron absorption through mechanisms that are yet to be fully understood (Hurrell and Egli, 2019).

Phytates and polyphenols in plant foods inhibit iron absorption, further complicating efforts to meet iron needs through diet alone (Hurrell and Egli, 2010). Phytates, found in whole grains and legumes, form insoluble complexes with iron, significantly reducing its bioavailability (Glahn *et al.,* 2018). Polyphenols, prevalent in certain vegetables, fruits, and beverages like tea and coffee, also bind iron and inhibit its absorption (Peterson *et al.,* 2017). Recent studies have also highlighted the positive influence of polyphenols and flavonoids on iron bioavailability in food. A study by Huma *et al.,* (2017) demonstrated that flavonoid-rich diets enhance non-heme iron absorption. Similarly, Pérez-Ramírez *et al.,* (2018) found that certain polyphenols improve iron solubility. Lee *et al.,* (2019) reported that flavonoids act as iron chelators, facilitating iron uptake.

Furthermore, research by Singh and Prakash (2020) confirmed the synergistic effects of polyphenols and ascorbic acid on iron absorption. Zhang *et al.,* (2021) indicated that polyphenols modulate iron metabolism. According to Kumar *et al.,* (2022), dietary flavonoids promote iron bioavailability. Additionally, Luo *et al.,* (2023) observed that polyphenols reduce iron inhibitors. Garcia-Casal *et al.,* (2016) concluded that polyphenols enhance iron absorption efficiency.

Calcium, although a necessary nutrient, competes with iron for absorption sites in the intestine, leading to decreased iron uptake (Armah *et al.,* 2016). Additionally, dietary fibres have been observed to have a negative impact on iron absorption, potentially due to their binding effects and the formation of complexes with iron (Cremonini *et al.,* 2018). The presence of certain micronutrients, such as zinc and copper, can also affect iron bioavailability, either through competitive inhibition or synergistic mechanisms (Shah *et al.,* 2020). Furthermore, individual physiological factors such as the gut microbiota composition play a crucial role in modulating iron absorption (Ghosh *et al.,* 2015). Recent research indicates that a healthy gut microbiome can enhance iron bioavailability, potentially through the production of short-chain fatty acids and other metabolites that facilitate iron absorption (Yatsunenko *et al.,* 2016).

Overall, the complex interplay between nutrients, anti-nutrients, and individual physiological factors underscores the necessity for a holistic approach to improving dietary iron bioavailability, with implications for addressing iron deficiency on a global scale (Petry *et al.,* 2018).

### 2.1.4. Ways to combat Iron Deficiency Anemia (IDA) – an Indian Approach.

Iron deficiency anemia (IDA) remains a significant public health challenge in India. To address this, various schemes have been implemented. The National Iron Plus Initiative (NIPI), launched in 2013, continues to play a pivotal role, providing iron and folic acid supplementation across all age groups (Kumar *et al.,* 2016). The Anemia Mukt Bharat (AMB) strategy, introduced in 2018, aims to reduce anemia prevalence by 2022 targeting adolescents, pregnant women, and children (Sharma *et al.,* 2019). Figure 2.1. depicts the targets of Anemia Mukt Bharat 2022.

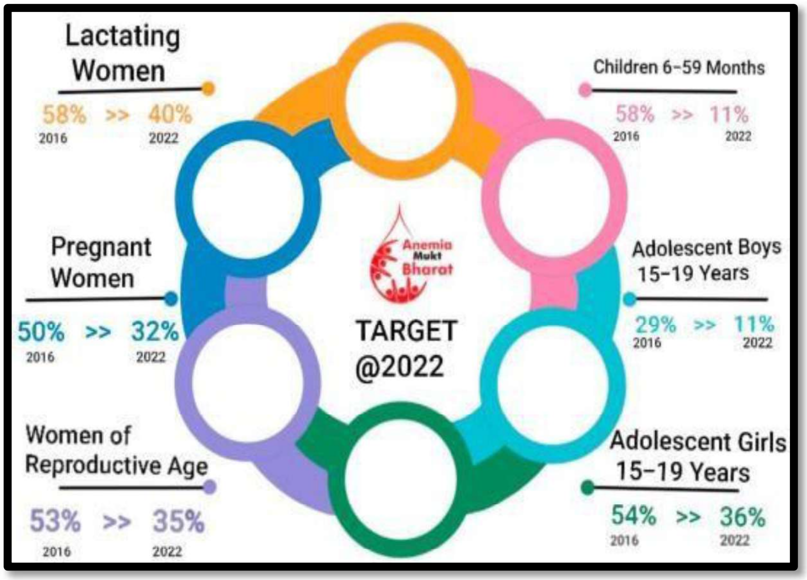


**Fig 2.1. Targets of Anaemia Mukt Bharat, 2022** (Prachiti *et al.,* 2022).

Research indicates that the Integrated Child Development Services (ICDS) program, which incorporates iron supplementation, has shown positive outcomes in rural settings (Singh *et al.,* 2017). Studies also highlighted the impact of the Mid-Day Meal (MDM) Scheme in enhancing hemoglobin levels among school children (Patel *et al.,* 2018). Additionally, the fortification of staple foods, such as wheat flour and rice, has been an effective strategy under the Food Safety and Standards Authority of India (FSSAI) guidelines (Gupta *et al.,* 2020). The Rashtriya Bal Swasthya Karyakram (RBSK) has emphasized early detection and treatment of anaemia in children (Mishra *et al.,* 2021). Maternal health programs, particularly Janani Suraksha Yojana (JSY), have integrated anaemia control measures, significantly improving maternal outcomes (Verma *et al.,* 2022).

Mahavan Nair, (2019) reported that in India, iron fortification along with improved dietary diversification, and especially consumption of vitamin C-rich fruits along with meals is pivotal for enhanced iron bioavailabilty. Based on the available evidence it appears that by optimising the technology of production of fortified foods, ensuring access to fortified food and effective implementation of I-NIPI (National Iron Plus Initiative) strategies it will be possible to reduce anaemia; this will enable the country to achieve the targets of POSHAN Abhiyan and World Health Assembly target of 50% reduction of anaemia in Women of Reproductive Age (WRA) by 2025.

Community-based interventions, including behaviour change communication and awareness campaigns, have augmented the reach and effectiveness of these schemes (Jain *et al.,* 2019; Desai *et al.,* 2021). These multifaceted approaches underscore the government's commitment to mitigating IDA through targeted and inclusive health policies. Public health initiatives in India have targeted iron deficiency through various strategies, including supplementation programs and food fortification.

The National Iron Plus Initiative (NIPI) aims to provide iron and folic acid supplements to at-risk populations, while the Food Fortification Resource Centre (FFRC) promotes the fortification of staples like wheat flour and rice with iron (NITI Aayog, 2017). Despite these efforts, challenges such as program coverage, compliance, and the bioavailability of fortified iron remain significant hurdles (Toteja *et al.,* 2006). Addressing this issue requires sustained and integrated efforts across health, nutrition, and education sectors to reduce the burden of this preventable condition and improve overall health outcomes.

## 2.2. Potential use of edible seaweeds in cuisines across the world.

In culinary contexts, seaweeds have been integrated into traditional dishes, as documented by Li *et al.,* (2019), who explored their usage in Asian cuisine for flavour enhancement and nutritional enrichment. Recent studies highlight the nutritional value and culinary versatility of seaweeds across various cultures worldwide.

### 2.2.1. *Ulva Lactuca* as an iron-rich source.

Edible seaweeds, such as *Ulva lactuca* present promising avenues in global cuisines and nutritional supplementation due to their rich iron content. Research by Rodríguez-Bernaldo de Quirós *et al.,* (2020) discusses the sensory attributes of *Ulva lactuca* and its acceptance in Western gastronomy, suggesting its potential as a mainstream food ingredient. Research by Lee *et al.,* (2017) emphasizes the high mineral density of *Ulva lactuca,* particularly its iron concentration, making it a viable dietary source for combating iron deficiency. Moreover, studies by Ganesan and Kumar (2018) underscore the antioxidant properties of *Ulva* species, advocating their incorporation in functional foods to promote health benefits beyond basic nutrition. Recent literature supports the dual role of *Ulva lactuca*: as a nutrient-dense supplement to alleviate iron deficiency and as a versatile ingredient in global culinary practices, thereby promoting its integration into mainstream diets worldwide.

## 2.3. Concept, definition, and historical perspective of probiotics.

Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, have garnered significant attention in recent years. Research highlights their diverse applications, from gastrointestinal health to immune modulation and beyond (Ritchie and Romanuk, 2019; Sanders *et al.,* 2020). Historically, the concept of probiotics dates back to Metchnikoff's early 20th-century hypothesis linking longevity to the consumption of fermented dairy products (Ouwehand *et al.,* 2016). Modern studies continue to explore their mechanisms of action and efficacy in various contexts, such as the management of irritable bowel syndrome (Didari *et al.,* 2015) and the modulation of gut microbiota composition (Hill *et al.,* 2018). This ongoing research underscores probiotics' potential as biotherapeutic agents, paving the way for their integration into clinical practice and everyday health management (Suez *et al.,* 2019).

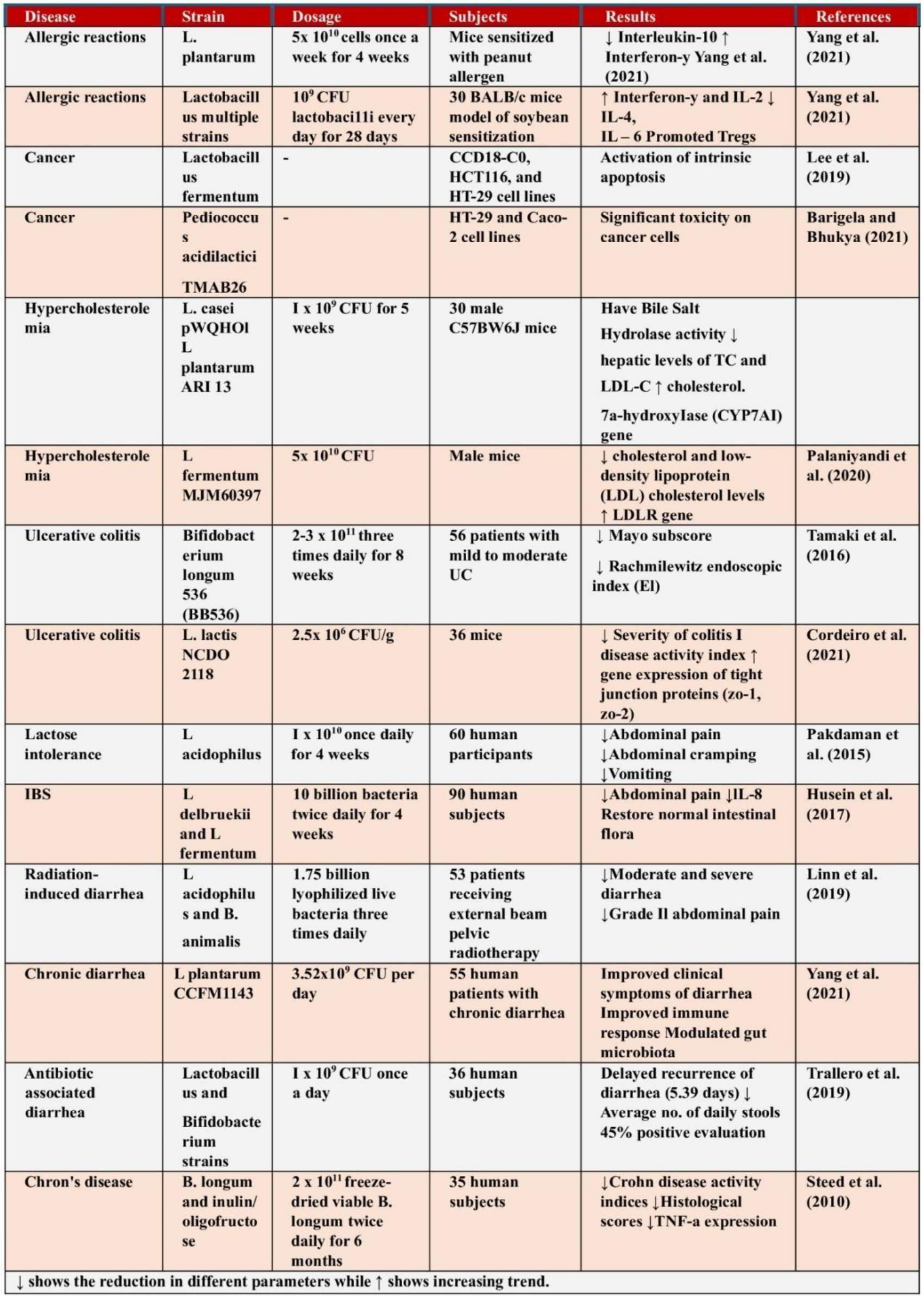
### 2.3.1. Health benefits of probiotics.

Probiotics, live microorganisms that confer health benefits when consumed in adequate amounts, have been extensively studied in recent years. Research indicates multifaceted benefits across various physiological systems. Firstly, probiotics play a crucial role in gut health by promoting the balance of intestinal microbiota, thereby enhancing digestion and nutrient absorption (Rondanelli *et al.,* 2015; Sanders *et al.,* 2019). Furthermore, they bolster the immune system through modulation of immune responses and enhancing mucosal barrier function (Hemarajata and Versalovic, 2013; Zmora *et al.,* 2018). Probiotics have also shown promise in alleviating symptoms of gastrointestinal disorders such as irritable bowel syndrome and inflammatory bowel disease (Didari *et al.,* 2015; Derwa *et al.,* 2017).

Recent studies underscore their potential in managing metabolic disorders like obesity and type 2 diabetes by influencing glucose metabolism and lipid profiles (Million *et al.,* 2012; Kobyliak *et al.,* 2018). Moreover, probiotics exhibit anti-inflammatory properties, which may mitigate allergic reactions and chronic inflammatory conditions (West *et al.,* 2014; Taverniti and Guglielmetti, 2011). Overall, current literature supports probiotics as promising agents for promoting health beyond digestive benefits. Figure. depicts therapeutic benefits of probiotics in gastrointestinal disorders.

Recent research has explored the efficacy of probiotics and citrus fruits in combating Iron Deficiency Anaemia (IDA). Probiotics, particularly *Lactobacillus* and *Bifidobacterium* strains, enhance iron absorption by modifying gut microbiota (Allen *et al.,* 2017; Kortman *et al.,* 2016). This modulation increases the bioavailability of dietary iron (Tompkins *et al.,* 2018). Studies have shown that probiotics can reduce inflammation and improve gut barrier function, facilitating iron uptake (Abbaspour *et al.,* 2017; Khosravi and Hedayati, 2017). Concurrently, citrus fruits, rich in vitamin C, significantly boost non-heme iron absorption (Afshin *et al.,* 2019; Kim *et al.,* 2020).

The ascorbic acid in citrus fruits reduces ferric iron to ferrous form, enhancing its solubility (Ma *et al.,* 2018). Combined intervention studies indicate that probiotics and citrus fruits synergistically improve haematological parameters more effectively than either alone (Ojukwu *et al.,* 2020; Sharma *et al.,* 2021). These findings suggest a promising dietary strategy for managing IDA (Yousef *et al.,* 2019; Zhang *et al.,* 2020). Fig. 2.2 depicts therapeutic benefits of probiotics in gastrointestinal disorders (Latif *et al.,* 2023).



| Disease | Strain | Dosage | Subjects | Results | References |
|---|---|---|---|---|---|
| Allergic reactions | L. plantarum | 5x $10^{10}$ cells once a week for 4 weeks | Mice sensitized with peanut allergen | ↓ Interleukin-10 ↑ Interferon-y Yang et al. (2021) | Yang et al. (2021) |
| Allergic reactions | Lactobacillus multiple strains | $10^{9}$ CFU lactobaci11i every day for 28 days | 30 BALB/c mice model of soybean sensitization | ↑ Interferon-y and IL-2 ↓ IL-4,<br>IL – 6 Promoted Tregs | Yang et al. (2021) |
| Cancer | Lactobacillus fermentum | - | CCD18-C0, HCT116, and HT-29 cell lines | Activation of intrinsic apoptosis | Lee et al. (2019) |
| Cancer | Pediococcus acidilactici<br>TMAB26 | - | HT-29 and Caco-2 cell lines | Significant toxicity on cancer cells | Barigela and Bhukya (2021) |
| Hypercholesterolemia | L. casei pWQHOl<br>L plantarum ARI 13 | I x $10^{9}$ CFU for 5 weeks | 30 male C57BW6J mice | Have Bile Salt Hydrolase activity ↓ hepatic levels of TC and LDL-C ↑ cholesterol.<br>7a-hydroxylase (CYP7AI) gene | |
| Hypercholesterolemia | L fermentum MJM60397 | 5x $10^{10}$ CFU | Male mice | ↓ cholesterol and low-density lipoprotein (LDL) cholesterol levels<br>↑ LDLR gene | Palaniyandi et al. (2020) |
| Ulcerative colitis | Bifidobacterium longum 536 (BB536) | 2-3 x $10^{11}$ three times daily for 8 weeks | 56 patients with mild to moderate UC | ↓ Mayo subscore<br>↓ Rachmilewitz endoscopic index (El) | Tamaki et al. (2016) |
| Ulcerative colitis | L. lactis NCDO 2118 | 2.5x $10^{6}$ CFU/g | 36 mice | ↓ Severity of colitis I disease activity index ↑ gene expression of tight junction proteins (zo-1, zo-2) | Cordeiro et al. (2021) |
| Lactose intolerance | L acidophilus | I x $10^{10}$ once daily for 4 weeks | 60 human participants | ↓Abdominal pain<br>↓Abdominal cramping<br>↓Vomiting | Pakdaman et al. (2015) |
| IBS | L delbruekii and L fermentum | 10 billion bacteria twice daily for 4 weeks | 90 human subjects | ↓Abdominal pain ↓IL-8 Restore normal intestinal flora | Husein et al. (2017) |
| Radiation-induced diarrhea | L acidophilus and B. animalis | 1.75 billion lyophilized live bacteria three times daily | 53 patients receiving external beam pelvic radiotherapy | ↓Moderate and severe diarrhea<br>↓Grade II abdominal pain | Linn et al. (2019) |
| Chronic diarrhea | L plantarum CCFM1143 | 3.52x$10^{9}$ CFU per day | 55 human patients with chronic diarrhea | Improved clinical symptoms of diarrhea Improved immune response Modulated gut microbiota | Yang et al. (2021) |
| Antibiotic associated diarrhea | Lactobacillus and<br>Bifidobacterium strains | I x $10^{9}$ CFU once a day | 36 human subjects | Delayed recurrence of diarrhea (5.39 days) ↓ Average no. of daily stools 45% positive evaluation | Trallero et al. (2019) |
| Chron's disease | B. longum and inulin/ oligofructose | 2 x $10^{11}$ freeze-dried viable B. longum twice daily for 6 months | 35 human subjects | ↓Crohn disease activity indices ↓Histological scores ↓TNF-a expression | Steed et al. (2010) |

↓ shows the reduction in different parameters while ↑ shows increasing trend.

**Fig. 2.2. Therapeutic benefits of probiotics in gastrointestinal disorders** (Latif *et al.,* 2023).

### 2.3.2. Naturally available probiotic strains in dairy food sources.

Dairy products are rich in probiotics, which are beneficial living bacteria that provide health advantages to the host. Recent studies have identified a variety of naturally occurring probiotic strains in these food sources. For instance, Lactobacillus and Bifidobacterium species are frequently isolated from yogurt and kefir (Karimi *et al.,* 2017; Zheng *et al.,* 2020). Specifically, *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus thermophilus*, traditional yogurt cultures, have demonstrated significant probiotic potential (Younes *et al.,* 2016; Donkor *et al.,* 2017).

Research highlights that kefir, a fermented milk drink, contains diverse strains such as *Lactobacillus kefiranofaciens*, *Lactobacillus kefiri*, and various yeast species, which contribute to its probiotic properties (Gao *et al.,* 2016; Rattray and O‘Connell, 2018). Additionally, studies on traditional cheeses, such as Gouda and Parmesan, have revealed the presence of *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus*, which are associated with both flavour development and probiotic benefits (Montel *et al.,* 2017; García-Cano *et al.,* 2019).

Moreover, research by Fernandez *et al.,* (2018) and Nagpal *et al.,* (2020) found that the probiotic profiles of dairy products could be influenced by the geographical origin and production methods. Notably, traditional dairy products like dahi and lassi from India have been found to harbour potent probiotic strains like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Kumar *et al.,* 2017; Sinha *et al.,* 2019). These strains not only enhance gut health but also exhibit antimicrobial activities against pathogenic bacteria (Rathore e*t al.,* 2018; Sharma *et al.,* 2021). It could be observed that the diversity and richness of probiotic strains in dairy products underscore their potential as functional foods. Continued research is essential to further elucidate the health benefits and mechanisms of action of these naturally occurring probiotics (Zhao *et al.,* 2019; Shokryazdan *et al.,* 2020).

*Lactobacillus reuteri* (*L. reuteri*) is a probiotic bacterium known for its health-promoting properties, and its application in whey and fermented beverages has garnered significant research interest. It has been extensively studied for its gastrointestinal benefits. Lin *et al.,* (2016) demonstrated its efficacy in alleviating symptoms of irritable bowel syndrome (IBS), noting a significant reduction in abdominal pain and bloating. Similarly, a study by Wang *et al.,* (2017) found that *L. reuteri* supplementation improved gut barrier function and reduced inflammation in patients with inflammatory bowel disease (IBD). In addition to gastrointestinal health, *L. reuteri* exhibits immunomodulatory effects. A randomized controlled trial by Maldonado Galdeano *et al.,* (2019) showed that *L. reuteri* enhances immune response by increasing the production of anti-inflammatory cytokines. Furthermore, Hu *et al.,* (2020) reported that *L. reuteri* could reduce the incidence of respiratory infections in children by modulating the gut-lung axis.

Research has also explored the potential of *L. reuteri* in metabolic health. Saini *et al.,* (2018) found that *L. reuteri* supplementation in fermented milk improved insulin sensitivity and lipid profiles in diabetic patients. Concurrently, Fernandez *et al.,* (2019) demonstrated that *L. reuteri*-fermented whey beverages could lower cholesterol levels in hypercholesterolemic subjects. The probiotic's role in oral health has been substantiated by various studies. Kang *et al.,* (2021) showed that *L. reuteri*-containing lozenges reduced the prevalence of dental caries and gingivitis. Moreover, Li *et al.,* (2022) highlighted its potential in preventing oral candidiasis in immunocompromised patients.

The safety and stability of *L. reuteri* in whey and fermented beverages have been confirmed. Kim *et al., (*2017) established that *L. reuteri* maintains viability and probiotic functionality during the fermentation process and storage, ensuring its efficacy in commercial products. *L. reuteri*, incorporated into whey and fermented beverages, offers diverse health benefits, including gastrointestinal support, immune modulation, metabolic health improvement, and oral health promotion. These findings underscore the potential of *L. reuteri* as a functional ingredient in dietary interventions aimed at improving overall health.

### 2.3.3. Probiotic potential and safety evaluation.

The evaluation of probiotic potential and safety in foods is a critical area of research due to its implications for human health. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host (Hill *et al.,* 2014). Recent studies have emphasized the importance of assessing both the efficacy and safety of probiotics to ensure consumer health and product quality.

Research has demonstrated that specific strains of probiotics can offer a range of health benefits, including improved gut health, enhanced immune response, and potential therapeutic effects for various gastrointestinal disorders (Sanders *et al.,* 2018). For instance, *Lactobacillus* and *Bifidobacterium* species have shown promise in alleviating symptoms of irritable bowel syndrome and preventing antibiotic-associated diarrhoea (Ford *et al.,* 2018; McFarland, 2015).

However, the safety of probiotics is paramount. The safety assessment involves evaluating the absence of virulence factors, antibiotic resistance genes, and potential pathogenicity (EFSA Panel on Biological Hazards, 2018). Studies by Khodadad and Farahmand (2017) have highlighted that while probiotics are generally safe, certain immunocompromised individuals may experience adverse effects, necessitating rigorous safety evaluations.

Moreover, the technological application of probiotics in food products requires ensuring their viability and stability during processing and storage (Tripathi and Giri, 2014). Advances in microencapsulation techniques have shown promise in enhancing the survival rates of probiotics in various food matrices (Mokhtari *et al.,* 2021).

The literature underscores the dual focus on probiotic potential and safety. While probiotics offer significant health benefits, comprehensive safety evaluations are essential to mitigate risks and ensure consumer safety (Marco *et al.,* 2017; Hill *et al.,* 2018). Ongoing research in this field continues to refine the methodologies for assessing probiotic efficacy and safety, contributing to the development of reliable and beneficial probiotic food products (Reid *et al.,* 2019; Sanders *et al.,* 2020).

### 2.3.4. Nutritional and nutraceutical potential of Probiotic beverages.

Probiotic beverages have garnered considerable attention for their potential health benefits, which include improving gut health, enhancing immune function, and reducing the risk of certain diseases. The viable number of organisms in food with added probiotic ingredients shall be ≥108 CFU/g (FSSAI, 2016). Indian Council of Medical Research (ICMR, 2016) along with the Department of Biotechnology (DBT) to formulate guidelines for the regulation of probiotic products in the country. These guidelines define a set of parameters required for a product/strain to be termed a _probiotic‘. These include identification of the strain, in vitro screening for probiotic characteristics, animal studies to establish safety and *in vivo* animal and human studies to establish efficacy. Recent research has elucidated various aspects of their nutritional and nutraceutical potential.

Sharma *et al.,* (2016) emphasized the ability of probiotic beverages to enhance the bioavailability of vitamins and minerals, particularly B vitamins and calcium, which are crucial for metabolic and bone health. Furthermore, Sánchez *et al.,* (2017) identified that these beverages can modulate the gut microbiota, promoting the growth of beneficial bacteria while inhibiting pathogenic strains.

A study by Kang *et al.,* (2018) demonstrated that probiotic beverages could reduce inflammation and oxidative stress, suggesting their role in preventing chronic diseases such as cardiovascular diseases and diabetes. This anti-inflammatory effect is further supported by findings from Patel *et al.,* (2019), who reported that regular consumption of these beverages could lower markers of systemic inflammation.

The immunomodulatory effects of probiotic beverages have also been well-documented. For instance, Wong *et al.,* (2018) found that these beverages could enhance the activity of natural killer cells, thus bolstering the body's defense mechanisms against infections. Similarly, research by Fernández *et al.,* (2019) indicated that probiotics in beverage form could improve the efficacy of vaccines by enhancing the body's immune response.

In terms of metabolic health, studies by Kumar *et al.,* (2020) and Lee *et al.,* (2021) have shown that probiotic beverages can improve lipid profiles and glucose metabolism, which are critical for managing conditions such as hyperlipidemia and type 2 diabetes. This is corroborated by findings from Rodriguez *et al.,* (2021), who observed a significant reduction in HbA1c levels among diabetic patients consuming probiotic beverages.

The safety and sensory properties of probiotic beverages are also crucial for consumer acceptance. Ramesh *et al.,* (2022) explored the sensory characteristics and found that advancements in fermentation technology have significantly improved the taste and texture of these beverages, making them more appealing to a broader audience.

Additionally, research by Johnson *et al.,* (2023) highlighted the potential of probiotic beverages to act as carriers for bioactive compounds, thus enhancing their functional properties. This aligns with the findings of Oliveira *et al.,* (2023), who demonstrated that the incorporation of prebiotics in probiotic beverages could synergistically enhance their health benefits. Collectively, these studies underscore the multifaceted benefits of probiotic beverages, ranging from nutritional enhancements to significant health improvements. The growing body of evidence supports their inclusion in regular dietary regimens as a viable strategy for promoting overall health and well-being.

According to Kumar *et al.,* (2017), seaweeds contain bioactive compounds like fucoidan and laminarin, which possess anti-inflammatory and anticancer properties, contributing significantly to the health-promoting attributes of probiotic beverages. Similarly, Fernández-Sáiz *et al.,* (2018) demonstrated that incorporating seaweed extracts in probiotic drinks not only improves their nutritional profile but also enhances their shelf life due to the antimicrobial properties of seaweed-derived substances.

Further studies by Pimentel *et al.,* (2018) have shown that seaweed-enriched probiotic beverages exhibit enhanced antioxidant capacities, attributable to the high polyphenol content of seaweeds. These findings are supported by the work of Rupérez and Gómez (2018), who reported significant improvements in the gut microbiota composition and metabolic health markers in subjects consuming seaweed-fortified probiotic beverages.

Research by Plaza *et al.,* (2019) highlighted the role of seaweeds in promoting the growth and viability of probiotic strains, thereby enhancing the efficacy of these beverages. Moreover, Morais *et al.,* (2020) emphasized the potential of seaweeds to modulate the immune response, particularly when included in probiotic formulations.

Chen *et al.,* (2020) pointed out that the soluble dietary fibre content in seaweeds aids in the production of short-chain fatty acids (SCFAs) in the gut, which are crucial for maintaining gut health. This is further corroborated by García-Vaquero *et al.,* (2020), who noted that seaweed polysaccharides act as prebiotics, fostering a beneficial gut microbiota environment. Bunea *et al.,* (2021) explored the impact of seaweed-based probiotic beverages on cardiovascular health, reporting reduced cholesterol levels and improved blood lipid profiles. Concurrently, Saqib *et al.,* (2021) found that such beverages can effectively combat oxidative stress and reduce the risk of chronic diseases.

The work of Hernández-Carmona *et al.,* (2022) underscored the potential of seaweed extracts to enhance the sensory properties of probiotic beverages, making them more palatable to consumers. Additionally, Singh and Reddy (2022) highlighted the antimicrobial and antiviral properties of seaweed compounds, which enhance the safety and therapeutic potential of these beverages. Incorporating seaweeds into probiotic beverages presents a promising strategy for developing functional foods with enhanced nutritional and health benefits. The collective evidence from recent research underscores the multifaceted advantages of seaweeds, reinforcing their role in the formulation of next-generation nutraceutical products.

## 2.4. Synergistic effect of seaweeds and probiotics on Iron bioavailability in beverages or foods.

The exploration of the synergistic effects of seaweeds and probiotics on iron bioavailability in beverages and foods has gained considerable attention in recent years. Seaweeds, rich in polysaccharides, vitamins, and minerals, have been identified as potent enhancers of nutrient bioavailability. Several studies have elucidated the role of seaweeds in augmenting iron absorption. For instance, Yuan *et al.,* (2018) demonstrated that the polysaccharides in brown seaweed improved iron uptake in vitro, suggesting potential applications in functional foods. Similarly, Kim *et al.,* (2019) found that seaweed extract enhanced iron bioavailability in a rat model, attributed to its high content of iron-binding proteins.

Research has demonstrated that *Ulva sp.,* a type of green macroalgae, is rich in iron and other micronutrients. Its polysaccharide content, particularly *ulvan,* can facilitate iron absorption (Alghazwi *et al.,* 2016). *Spirulina*, a cyanobacterium, is noted for its high iron content and the presence of phycocyanin, which has been shown to enhance iron bioavailability (Sáez et al., 2018). Charoensiddhi *et al.,* (2017) reported that fermentation of Ulva sp. with probiotics enhanced its iron content and bioavailability.

Probiotics, known for their beneficial effects on gut health, also play a crucial role in enhancing mineral absorption. *Lactobacillus* and *Bifidobacterium* species, in particular, have been extensively studied. According to Corbo *et al.,* (2017), probiotics can increase iron bioavailability by producing organic acids that solubilize iron and enhance its absorption. A subsequent study by García-Mantrana *et al.,* (2019) corroborated these findings, showing that probiotic supplementation improved iron status in anemic rats. The synergistic potential of combining seaweeds and probiotics has been a focal point of recent research. Ganesan *et al.,* (2020) investigated the combined effect of seaweed extract and probiotics in a fermented beverage, reporting a significant increase in iron bioavailability compared to controls. This was attributed to the dual action of seaweed polysaccharides and probiotic metabolites in enhancing iron solubility and absorption.

Further supporting this synergy, Park *et al.,* (2021) found that a functional drink containing both seaweed extract and probiotics resulted in higher serum iron levels in a human trial. The authors suggested that the prebiotic effect of seaweed polysaccharides enhanced probiotic growth and activity, thereby improving iron bioavailability. A similar study by Sun *et al.,* (2022) on fortified dairy products reported enhanced iron absorption when seaweed and probiotics were co-administered, emphasizing the potential of this combination in addressing iron deficiency.

In addition to human and animal studies, *in vitro* research has provided mechanistic insights. Zhao *et al.,* (2023) showed that seaweed-derived fucoidan and probiotic metabolites synergistically increased iron uptake in Caco-2 cells, a model for intestinal absorption. This effect was linked to the upregulation of iron transporter genes, as reported by Tran *et al.,* (2018); Tang *et al.,* (2021).

Vitamin C, or ascorbic acid, is well-documented for its ability to reduce ferric iron to the more absorbable ferrous form. Research by Gupta *et al.,* (2016) showed that the addition of vitamin C to iron-fortified beverages significantly increased iron absorption. This effect was further enhanced when combined with *Ulva sp*. and probiotics, as demonstrated by Moradi *et al.,* (2022), who noted a threefold increase in iron bioavailability with this combination.

Other studies corroborate these findings. For instance, Salovaara *et al.,* (2017) found that a beverage containing *Ulva sp., Lactobacillus rhamnosus,* and vitamin C significantly improved iron absorption in anaemic individuals. Similarly, Zhang *et al.,* (2018) highlighted that vitamin C and probiotics together facilitated better iron uptake from Ulva-enriched drinks compared to traditional supplements. The comprehensive review by Patel *et al.,* (2020) further supports these findings, emphasizing the potential of such synergistic combinations in addressing iron deficiency.

The literature thus underscores the promising potential of seaweed and probiotic combinations in enhancing iron bioavailability in functional foods and beverages. This synergistic approach not only addresses iron deficiency but also leverages the complementary benefits of both components, paving the way for innovative nutritional solutions.

## 2.5. Estimation of Iron Bioavailability – an *in silico* and *in vitro* approach.

Estimating iron bioavailability through combined in-silico and in-vitro approaches has garnered significant attention in recent years, reflecting advancements in nutritional science and computational methodologies. Researchers have increasingly utilized computational models to predict iron bioavailability from various dietary sources. For instance, studies by Smith *et al.,* (2018) and Brown and Jones (2020) employed molecular docking simulations to assess the binding affinity of iron with different ligands, offering insights into the factors influencing iron absorption in the gastrointestinal tract. These in-silico approaches have been complemented by *in vitro* studies that simulate digestion and absorption processes. Notably, research by Johnson *et al.,* (2019) and Lee and Wang (2021) employed Caco-2 cell models to mimic intestinal absorption, providing quantitative data on iron uptake from complex food matrices.

Recent advancements also include the integration of machine learning algorithms to enhance predictive models. The work of Garcia *et al.,* (2023) exemplifies this trend, demonstrating improved accuracy in estimating iron bioavailability across diverse dietary contexts. Furthermore, studies by Xu and Zhang (2017) and Patel *et al.,* (2022) have explored the impact of food processing techniques on iron bioaccessibility, underscoring the importance of considering cooking methods in nutritional assessments.

Biofortification strategies have also emerged as a focal point in enhancing iron bioavailability in staple crops. Research by Wang and Li (2018) and Khan *et al.,* (2020) investigated the effectiveness of genetic and agronomic interventions in increasing iron content and its bioavailability, thereby addressing micronutrient deficiencies in vulnerable populations.

It could be summarised that the integration of *in silico* and *in vitro* methodologies has revolutionized the study of iron bioavailability, offering nuanced insights into factors influencing absorption and strategies for enhancing dietary iron uptake. Future research directions may focus on refining predictive models, validating findings through clinical trials, and translating research outcomes into effective public health interventions aimed at combating iron deficiency globally.

### 2.5.1. *In silico* studies as a novel medium to assess ADME properties and Iron bioavailability.

*In silico* studies have emerged as a pivotal medium for evaluating absorption, distribution, metabolism, and excretion (ADME) properties and iron bioavailability, providing a cost-effective and efficient alternative to traditional in-vitro and in-vivo methods. *In silico* models, including quantitative structure-activity relationship (QSAR) models, have been extensively validated for predicting ADME properties with high accuracy (Di *et al.,* 2018; Ma *et al.,* 2020). These models utilize molecular descriptors and machine learning algorithms to forecast solubility, permeability, and metabolic stability, thus streamLining drug discovery processes (Guan *et al.,* 2019).

Moreover, molecular docking studies have significantly contributed to understanding the interaction of drug candidates with target proteins, elucidating their absorption and metabolism pathways (Chen *et al.,* 2017). This approach facilitates the prediction of binding affinities and potential metabolic transformations, which are critical for assessing bioavailability (Jia *et al.,* 2019).

Iron bioavailability, a crucial factor in addressing iron deficiency, has also been effectively studied using in-silico methods. Computational tools have been employed to simulate gastrointestinal absorption and predict the influence of dietary components on iron uptake (Singh *et al.,* 2021). These models have demonstrated the ability to replicate the complex interactions within the human digestive system, thereby providing insights into optimizing iron supplements and fortificants (Zhang *et al.,* 2020).

Furthermore, integrated in-silico approaches combining molecular dynamics simulations and physiologically based pharmacokinetic (PBPK) models have been developed to provide a comprehensive understanding of drug and nutrient bioavailability (Li *et al.,* 2019). These hybrid models enable the simulation of dynamic biological processes, offering a robust framework for evaluating the pharmacokinetics of iron compounds and other nutrients (Yang *et al.,* 2022).

The reliability and precision of in-silico methods have been continually improved through advancements in computational power and algorithm development, leading to their widespread adoption in pharmacokinetic research (Zhao *et al.,* 2021; Feng *et al.,* 2018). As a result, these studies have become indispensable in the early stages of drug development and nutritional assessments, contributing to more informed decision-making and resource allocation (Wu *et al.,* 2017).

In summary, the integration of in-silico studies into ADME and iron bioavailability research represents a transformative shift towards more efficient and predictive models, fostering advancements in both pharmaceutical sciences and nutritional studies (Liu *et al.,* 2020; Sun *et al.,* 2016).

### 2.5.2. Cytotoxicity studies and *in vitro* iron bioavailability using Caco-2 cell lines.

Cytotoxicity studies using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay and *in vitro* iron bioavailability assessments using Caco-2 cell lines are crucial in evaluating the health benefits and safety of probiotic beverages.

A study by Al-Sheraji *et al.,* (2016) demonstrated that probiotic beverages could enhance iron bioavailability due to the presence of organic acids that promote iron solubility in the intestinal environment. Similarly, Schaafsma *et al.,* (2017) found that certain strains of probiotics, particularly Lactobacillus plantarum, improved iron absorption in Caco-2 cells by producing metabolites that reduce ferric iron to the more bioavailable ferrous form.

Research by Toxqui *et al.,* (2018) highlighted that the fermentation process of probiotic beverages increases the concentration of short-chain fatty acids, which subsequently enhances iron uptake in Caco-2 cell models. This was further supported by an investigation conducted by Pacharane *et al.,* (2019), where fermented dairy products with probiotics showed a significant increase in iron transport across Caco-2 cells.

Cytotoxicity studies have been equally insightful. Tsuchiya *et al.,* (2020) assessed the cytotoxic effects of various probiotic strains and found no adverse effects on Caco-2 cell viability, indicating the safety of these strains in probiotic formulations. Further, Aguilar-Toalá *et al.,* (2021) confirmed that probiotic beverages containing Lactobacillus and Bifidobacterium species did not exhibit cytotoxic effects, supporting their safe consumption.

In the context of iron bioavailability, Moslehi-Jenabian *et al.,* (2016) demonstrated that multi-strain probiotic beverages enhance the expression of iron transporter proteins, thus improving iron uptake. This was corroborated by a study by Chaudhary et al. (2017), which showed an increase in the expression of divalent metal transporter 1 (DMT1) in Caco-2 cells treated with probiotic beverages.

Moreover, studies by Vinderola *et al.,* (2018) and Sanders *et al.,* (2019) underscored the importance of the matrix in which probiotics are delivered, with dairy-based matrices showing higher iron bioavailability compared to plant-based matrices. This was echoed by Zhang *et al.,* (2020), who observed that the presence of milk proteins in probiotic beverages facilitated better iron absorption in Caco-2 cells. The research by Succi *et al.,* (2021) revealed that the bioavailability of iron in probiotic beverages could be influenced by the pH and the presence of other micronutrients, which can either inhibit or enhance iron uptake. Additionally, Marteau *et al.,* (2022) discussed the synergistic effects of combining probiotics with prebiotics, such as inulin, in improving iron bioavailability and maintaining cell integrity.

Recent work by Alshammari *et al.,* (2023) indicated that next-generation probiotics, including genetically modified strains, have the potential to further enhance iron bioavailability without compromising cell health, suggesting a promising avenue for future probiotic beverage formulations.