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## CHAPTER I

### INTRODUCTION

The Sustainable Developmental Goals 2 (SDGs) of 2015 aims to achieve food security and eradicate all types of hunger by 2030. Goal 2 of the SDGs commits to ensuring that everyone has access to safe, nourishing, sufficient and good quality food throughout the year for leading a healthy lifestyle. One in four young children under the age of five still suffers from chronic undernutrition, or stunted growth, which raises the risk of diseases like pneumonia, fever, dengue, diarrhoea, and malaria as well as the frequency and severity of infections and delays healing. Children who are underweight, especially those who have severe acute malnutrition, falls in the range of higher risk of death from common childhood diseases such pneumonia, malaria, diarrhoea etc. (<https://unstats.un.org/sdgs/report/2016/goal-02/>). The powerful proverb that "the children of today are the future of tomorrow" has additional significance in our context because children make up one third of the overall population of the nation. In India, National Family Health Survey-5 (2019-21) reported that 27.3% children in urban and 33.8% children in rural under five years of age are underweight (weight-for-age).

To achieve such SDGs, food diversifications places a significant place in Indian context and strategies like food fortification that is proven to be safe and economical, improvement of diets and to prevent micronutrient deficiencies. Food fortification was ranked by the Copenhagen Consensus as one of the most cost-effective development priorities that can be employed in long term basis to prevent hunger, starvation and micronutrient deficiencies (Olson *et al.*, 2021). These issues are best approached through the development of enriched and enhanced products like probiotic complementary food mixes from locally available raw ingredients for increasing the profitability, convenience and accessibility for vulnerable segment of our society as well as for lowering the primary risk factors for non-communicable diseases and lifestyle disorders. The main role of diet is to supply the required nutrients to meet metabolic requirements, also provide the consumer a feeling of satisfaction and well-being. Recent studies support the concept that diet may influence many physiological activities and may have

positive or therapeutic effects in specific disorders, in addition to providing nutritional demands. The concepts in nutrition sciences are evolving from the traditional knowledge of emphasis on survival, satisfying hunger, and avoiding negative effects to an emphasis on the utilization of foods to create a state of well-being, improve health, and lower the risk of various life style diseases (Granato *et al.*, 2010).

The right nutrition and diet are crucial for a child's healthy development and immune system. To improve children's nutrition, food systems must provide healthy, accessible, consistent diets for all children. Protein energy malnutrition is most common type of malnutrition in young children, which typically takes place during the important transitional period when infants are weaned from liquid (such as breast milk) to semi-solid or completely adult (family) diets. Malnutrition has a complicated pathophysiology, but one of the main causes is low food intake. After six months, a baby's nutritional and energy requirements start to surpass those of breast milk, necessitating the consumption of complementary foods. Hence infants are physically ready for foods other than breast milk after six month of age. This shift from breast milk to complementary foods is called as complementary feeding.

In order to assure that nutritional intakes remain adequate for optimal growth and development throughout childhood, complementary feeding is done. It is a process by which an infant proceeds from a diet consisting primarily of breast milk or commercial formula to a family diet comprising of diverse foods (WHO, 2003). Complementary foods are terms used to describe foods made from processed grains generally including cereals, millets legumes, nuts, and/or edible oilseeds that have been dried to low moisture content for longer shelf life (BIS,2006).Complementary foods as a whole plays a critical part in infant's physical maturation as it accompanies the nutritional as well as growth requirements of the infant once feeding from mother alone is not adequate for their growth (Temesgen, 2013).In addition to mother's milk, infants require nutritionally rich additional foods that are calorie-dense and nutritionally complete to support the body's growing needs (Abeshu *et al.*, 2016). High energy, protein, and micronutrient levels combined with low viscosity, bulk density, suitable texture

and acceptable consistency promotes consuming of complementary food mixes easily by the children. One of the major issues in developing nations like India are that majority of complementary food mixes fed by infants are lacking in major macronutrients and micronutrients, which are one of the leading cause of malnutrition (Balasubramanian *et al.*, 2014).

As breast milk contains all the nutrients that new borns need to grow the World Health Organization (WHO) made a global recommendation in 2021 to expand the period of exclusive breastfeeding from the previous advice of four to six months to a full six months. However, beyond six months of age, breast milk may not meet the nutritional and physiological needs of developing infants, so it is necessary to supplement breast milk with other meals after six months of ageso that it can assist to correct any deficiencies that may arise from such inadequacy (Ikujenlola and Adurotoye, 2014). In low income and developing countries, cereals are the base for development of complementary food mixes resulting in energy density lower than that suggested by World health organization (Perlas 2013)

The conventional complementary food mixes prepared with cereals like rice and wheat are basically based on staples that are easily and locally available. Essential amino acids such as lysine and tryptophan that are necessary for a children's healthy growth are lacking in homemade food mixes prepared only with cereals. Areas where consumption of cereals is predominant in infant feeding generally lacks in the quality of protein. According to the amino acid profiles of cereals, lysine and tryptophan are typically deficient while methionine and cysteine, two amino acids that contain sulphur, are present in appropriate amounts. To complement the amino acids of cereals in complementary food mixes, lysine which is present in reasonably proportion in pulses and legumes can be mixed in appropriate amount to balance the amino acids profile. The legume and pulses foods acts as a natural complements to cereal based diets for improving the chemical and nutritive characteristics (Ikujenlola and Fashakin, 2005; Abiose *et al.*, 2015). Hence, when legumes and cereals are blended in the proper ratios, the amino acids in the two foods complement one another further improving the protein content (Ghasemzadeh and Ghavidel 2011).

In majority of developed nations, expensive nutrient-fortified cereals and commercial fortified formulas are given to infants followed by fruits and vegetables juices and puree that are generally not accessible for groups that belong to lower income families. One feeding alternative that has been suggested is the use of home-based complementary food that is readily available, economical, and easy to prepare in order to lessen the impact of malnutrition on infants and young children (Akinola *et al.*, 2014). Numerous researchers have recommended using high nutrient dense food items such cereals and millets, legumes, oil seeds, fruits, vegetables to produce complementary mixes for children above six months of age. Different types of plant based mixes which are both nutritious and economical are given to boost the health status of children (Ikujenlola and Fashakin, 2005; Bala *et al.*, 2014). In the majority of the world's countries, cereals, millets, and pulses are significant crops utilised for human's survival. Because they are produced in major amounts, they function as significant provider of nutrients, phytonutrient, and other biologically active compounds. Cereals, millets, and legumes are typically pre-processed through milling, roasting, fermenting, germinating, and grinding etc. to optimize functionality and nutritional value.

Rice is the most recognized and important cereal in the world for many years and is currently even more in trend due to its numerous health benefits. FAO/WHO (2021) estimated production of 520.8 million tonnes of rice which accounted for half the global production of primary crops along with three other crops. The Department of Agriculture, Cooperation and Farmers Welfare in the year 2020-21 (<https://www.fao.org/worldfoodsituation/csdb/en/>) had announced that a total of 305.44 million tonnes of food grains were expected to be produced; of which 121.46 million tonnes were rice. The majority of developing nations rely heavily on rice as a staple food and main cereal crop. Next to China, India is the country that produces the second-most rice. Rice and its products are one of the major foods included in the diet by most of the Eastern countries especially India. Rice plays versatile uses from complementary baby foods to food multi mixes. The amount of calories for rice is same as that of counterpart wheat. Over half the world's population consumes rice since it is a primary food and is a significant

source of carbohydrates. Additionally, rice offers thiamin, riboflavin, niacin, and zinc in nutritionally significant levels, also with lower quantities of other micronutrients (Rathna *et al.*, 2019).

According to FAO (2020), production of millet worldwide was estimated to be 28.2 million metric tons in 2019, which had increased to 30.5 million metric tons in 2020. India is the largest global producer, with a 33.3% global market share in 2020 (<https://www.fao.org/worldfoodsituation/csdb/en/>). Globally the share for finger millet production is about 12.5% of the total millet (Gebreyohannes *et al.*, 2021). Millets are super foods that have nutrients including copper, magnesium, phosphorus, and manganese. Millets significant nutritious content aids in preserving a healthy lifestyle. Fiber found in millets aids in digestion and prevents intestinal problems. Millets aid in preventing gastrointestinal issues as well as various renal and liver ailments when consumed regularly. On the global scale, finger millet ranked fourth position among millets, next to sorghum, pearl millet and foxtail millet (Maharajan *et al.*, 2021). Also known as ragi, finger millet (*Eleusine coracana*) is a major source of calcium and iron. It is usually used to prepare flour, pudding, porridge, Indian bread and traditional complementary foods (Chaturvedi and Srivastava, 2008). Finger millet is one of the richest sources of nutrients compared to other minor cereals like sorghum, pearl millet etc. Finger millet is used for feeding infants since ancient times. The calcium content in finger millets is threefold the calcium found in milk along with it has prominent levels of iron and zinc with low glycemic index, high protein and fibre content, and are gluten-free. Millets can also help to address some of the world's most pressing problems such as poor diet from undernutrition to overnutrition, to habitat difficulties including degradation of nature and poverty. Minor millets like finger millet are recognized as important substitutes for major cereals like wheat and rice. They are claimed to be future foods for better health and nutrition security (Vanithasri *et al.*, 2012).

The International year of millets 2022-23 was announced in India to encourage the post-harvest value addition of millets, increasing local consumption and branding millet commodities domestically and globally. Millets are also known as Smart Food since they are beneficial to consumers, the environment, and

farmers. Finger millet food mixes are generally recommended for children as it is easily digestible, non allergic, non acidic forming foods (Saxena *et al.*, 2018).

Zinc insufficiency is a global issue and is responsible for more than 500,000 baby and young child deaths worldwide under the age of five (WHO, 2003). Although the amount of zinc transmitted from the mammary gland to the breastfed infant decreases as lactation advances, the average fractional uptake of zinc from breast milk is about 50%. The amount of zinc in breast milk declines significantly from colostrum to mature milk from around 4 mg per day in the first postpartum days to about 0.7 mg per day till six months, leading to speculation that zinc may be the first vitamin to reach a limiting level in breast milk (Maxfield *et al.*, 2021). Infants under six months of age may already be consuming less zinc from breast milk than is recommended. Developing countries in Asia has the highest rate of paediatric mortality from pneumonia, malaria, and diarrhoea due to zinc deficiency (Aumeistere *et al.*, 2018). For children under the age of five, diarrhoea is the third leading cause of under nutrition, mortality, and morbidity, particularly in developing nations like India. According to the World Health Organization's most recent study (WHO, 2020), diarrhoeal disease is the leading cause of death for children under the age of five in underdeveloped nations. Thus, it is evident that this disease is a significant public health concern in India (Ugboko *et al.*, 2020). According to the International Children's Emergency Fund of the United Nations in 2021, malnourishment (45 percent), pneumonia (15 percent), diarrhoea (8 percent), and malaria (5 percent) are the main causes of death in children under the age of five. Together, India, Nigeria, Congo, Pakistan, and China account for half of the 4.249 million children who died from diarrhoea in 2018. According to the National Family Health Survey (NFHS-5), 7.7 percent of children in rural regions and 6.2 percent of children in urban areas reported having diarrhoea in the year 2019–21. World Health Organization (WHO, 2001) defined diarrhoea as "three or more loose or watery stools in a 24 hour period." While persistent diarrhoea lasts 14 days or longer, acute diarrhoea lasts less than 14 days.

Scientific interest and knowledge are growing in the food industry about the use of microbial food additives in improving productivity of foods with functional ingredients and products. The last two decades have seen considerable development in probiotics which have revolutionized the food industry (Ouwehand *et al.*, 2002). FAO/WHO (2011) also defines probiotic as live microorganisms which confer health benefit to consumer when ingested in adequate quantity. Probiotics are viable microbial dietary supplements that affect the host beneficially through its effects in the intestinal tract. Metchnikoff from Pasteur Institute in the early 1900s was the first one to observe the positive role of these beneficial bacteria (Mackowiak, 2013). The consumption of probiotic alters the gastrointestinal flora in a manner which directly or indirectly provides health benefit to the host (Eckburg *et al.*, 2005). The possibility of fermented food products for lowering or alleviating food related aspects of malnutrition, particularly in weaning age children, is significant considering the health benefits these foods naturally provide (Wakil and Kazeem, 2012). Around the world, cereals are excellent substrates for fermentation, with lactic acid, bacteria, and yeasts being the most common microorganisms (Blandino *et al.*, 2003; Franz *et al.*, 2014). Lactic acid bacteria typically prevail over other flora as a complex microflora that forms spontaneously plays a major role in fermentation. Since spontaneous and natural fermentations are susceptible to pathogen contamination, they can be unsafe at times and are difficult to control. Therefore, in order to achieve a consistent level of product quality, the current uncontrolled fermentation technique used in developing nations must be replaced with pure culture fermentation (Semwal *et al.*, 2015). Gram positive, anaerobic, non-sporulating, acid tolerant lactic acid bacteria are used in developing food and food products because they are usually recognised as safe (GRAS). They also contribute to flavour, texture, and nutrition as well as the quick acidification of food products. Fermentation with the help of Lactic acid bacteria (LAB) improves digestibility and reduces diarrhoea illness in infants (Mathur *et al.*, 2020).

There has been an expanding tendency in recent years for consuming probiotic foods with functional properties additional to their nutritional value (Lourens-Hattingh, 2001). Functional foods are ones that have physiological

advantages and can lower the risk of chronic diseases in addition to serving fundamental nutritional purposes, such as maintaining gut health (FAO, 2019). Increasing customer vegetarianism, lactose intolerance, cholesterol content, and economic factors linked to dairy products are driving the adoption of cereal-based substrate for the creation of probiotic products and food items (Prado *et al.*, 2008; Gobbetti *et al.*, 2010). Additionally, cereals may help consumers by providing prebiotics and whole grains (Lamsal and Faubion 2009). Few studies had been reported on the suitability of cereals as carriers of probiotic lactic acid bacteria, but none have examined the use of probiotic strains to create cereal-based probiotic foods, despite studies supporting the probiotic potentials and the substantial physiological diversity of traditional fermented cereal-based foods. The majority of the health effects attributed to probiotic are either directly or indirectly related to the gastrointestinal tract, using them to treat or prevent gastrointestinal disorders like diarrhoea, irritable bowel syndrome, or celiac disease is perhaps the most common use of probiotic (Markowiak and Śliżewska, 2017).

Numerous factors, such as child malnutrition, low income groups and illiteracy among mothers, lack of access to hygienic drinking water, poor hygiene or overpopulation continue to place a significant burden on children in under developed and developing nations. The factors stated above are also strongly related to diarrhoeal disease for maximum children. Poor hygiene practices, particularly in the preparation of homemade complementary food mixes due to costly commercial mixes may increase the risk of diarrhoea and are responsible for up to 70% of diarrhoea episodes (Oloruntoba *et al.*, 2014). Breast milk along with complementary food mixes have to be provided to an infant after six months of age. Complementary foods mixes when introduced to the infants in many nations are prepared under unhygienic circumstances; infants till then had only consumed mother's milk may be subjected to toxic elements present in the food leading to food borne diseases. Researches had proven that after weaning begins for infants, the frequency of diarrhoeal illnesses is particularly high (D'Auria *et al.*, 2020).

By replenishing and maintaining the good bacteria in the gut and reversing an imbalance, probiotic supplements may help treat diarrhoea and prevent some

types of it. By competing for nutrients, enhancing the immune system, and altering the gut environment by making it less favourable for pathogenic activity, probiotic bacteria combat pathogenic bacteria (Allen *et al.*, 2010). Probiotics primarily serve as supplements to the infant's GI tract microbiota and provide clinical advantages with relation to a number of illnesses and medical disorders. Thus, utilising the benefits of probiotics as a food ingredient is a wise course of action to take in order to prevent illnesses and enhance health (Salminen *et al.*, 2021). Probiotics with a long history of safety, including *Lactobacillus spp.* and *Bifidobacterium bifidum* have been found to prevent retroviral diarrhoea. However, they must be added to food to ensure their bioavailability if they are to have a therapeutic impact. To add probiotics to a baby's diet for long-term usage in preventative healthcare, however, is advised through fermented dairy foods, infant formula, or any other types of food supplements ingested during the weaning process. This is due to the fact that this approach is more realistic and aids in both cost-reduction and the creation of compliance initiatives. A daily dose of probiotics with  $10^8$  - $10^{10}$  CFU is required to have a therapeutic and physiological effect (Donnet-Hughes *et al.*, 1999). The delivery system and the process used for preparing probiotic products can be used to determine the quantity of live bacteria that enter the target organ. Thus, it can be said that the amount of probiotics needed varies depending on the species being used and the host organism. Keeping the above facts in mind, the present research study entitled “**Assessment of nutritional and functional properties of probiotic complementary food mixes from locally available cereals and legumes**” aims to develop suitable food formulations utilizing cereals/millet, pulses, and oil seeds and fermenting it with probiotic strains for developing them for household purpose and commercial exploitation with the following hypothesis and objectives:

#### **Hypothesis of the Study:**

- $H_0$  - The Probiotification of complementary food mixes will not significantly improve the functional and nutritional quality of mixes.
- $H_0$  -Supplementation of developed probiotic complementary food mixes to Wistar albino rats will not significantly reduce the diarrhoeal episodes of experimental rats.

- $H_0$  -The probiotic complementary food mixes cannot be significantly stored for more than 90 days at ambient temperature.

**General objectives of the study:**To

- Assess the nutritional and functional properties of probiotic complementary food mixes from locally available cereals and legumes

**Specific Objectives of the study:** To

- Develop probiotic complementary food mixes from locally available cereals and legumes
- Assess the physico-chemical and functional properties of the developed complementary food mixes
- Study the shelf life properties of the developed complementary food mixes
- Evaluate the *In-vivo* efficacy of developed probiotic complementary food mixes through animal model.

**Scope of the study:**

- Achieve Sustainable Development Goal 2's target of 2030 for the eradication of all types of hunger and malnutrition. Additionally, it makes a commitment to ensuring that everyone has access to adequate, nutritious food at all times of the year, as determined by the global assembly.
- An ideal and novel probiotified complementary food mixes for enhancing both physical and mental development of children (6-24 months).
- A staple based food mixture apart from dairy based developed from the commonly used cereals and legumes fermented with a probiotic microorganism having better profile of nutrients and therapeutic value
- Efforts to include low cost nutritious complementary food mixes from locally available cereals and legumes into the government schemes and policies for sustainable food security.