CHAPTER IV

RESULTS AND DISCUSSION

The results of the present investigation titled "**Assessment of nutritional and functional properties of probiotic complementary food mixes from locally available cereals and legumes**" are presented and discussed under the following headings:

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PHASE I

4.1. Nutrient composition of functional ingredients used for formulation of complementary food mixes

4.1.1. Pre and post effect of processing on the proximate principle composition of raw ingredients used for formulation of complementary food mixes

Consumers consume different varieties of food products consisting of chemically diverse compounds. Hence the idea of 'functional foods' came into origin. Consumer interest in the health promoting properties of specific foods or physiologically active food components, sometimes known as functional foods, has exploded recently. A food containing dietary component may or may not be a nutrient which targets one or more functions in human body, to produce favourable results to support particular health claims causing positive health effects.

Table 4.1 gives thepre and post effect of processing on the proximate principle composition of raw ingredients identified the difference in the macronutrients contents in the raw ingredients or the food samples.

Table 4.1. Pre and post effect of processing on the principle composition of raw ingredients used for formulation of complementary food mixes

Functional ingredients	Nutrients (per 100 g of dry weight basis)														
	Moisture (g)			Available carbohydrate (g)			Crude protein (g)			Fat (g)			Crude fibre (g)		
	Before	After	Improve-ment	Before	After	Improve-ment	Before	After	Improve-ment	Before	After	Improv ement	Before	After	Improve ment
Rice flour (*Oryza sativa*)	11.20 ±0.01	3.46 ±0.06	-7.74	72.04± 0.05	82.94 ±0.005	10.9	5.85 ±0.01	6.00± 0.005	0.15	1.13± 0.02	3.14± 0.38	2.01	2.05± 0.01	3.05±0. 001	1.00
Pearl millet flour (*Pennisetum glaucum*)	11.20 ±0.05	3.90± 0.10	-7.30	70.00± 0.07	75.00± 0.010	5.00	10.40±0. 50	11.40± 0.005	1.00	3.80± 0.14	4.08± 0.14	0.28	1.88± 0.015	3.49± 0.001	1.61
Finger millet flour (*Eleusine coracana*)	5.58± 0.20	3.50± 0.10	-2.08	74.03± 0.05	88.50± 0.015	14.47	7.42± 0.05	7.80± 0.001	0.38	6.20± 0.06	1.20± 0.05	5.00	2.50± 0.06	3.65± 0.005	1.15
Malted wheat flour (*Triticum aestivum*)	11.01± 0.15	4.01± 0.10	-7.00	63.86± 0.005	55.86± 0.01	8.00	10.50± 0.03	30.00± 0.005	19.50	9.80± 0.002	11.05±0. 05	1.25	3.12± 0.001	3.50±0. 05	0.38
Mung bean flour (*Vigna radiata*)	10.00± 0.05	3.36± 0.10	-6.64	54.09± 0.05	73.00± 0.005	18.91	20.02± 0.05	23.32± 0.001	3.30	1.05± 0.01	1.48± 0.25	0.43	2.05± 0.06	3.91± 0.005	1.86
Soyabean flour (*Glycine max*)	7.05± 0.10	5.02± 0.10	-2.03	30.10± 0.06	19.08 ±0.005	11.02	34.06± 0.10	39.28± 0.005	5.22	19.08± 0.01	10.48± 0.13	8.6	4.00± 0.50	4.91± 0.001	0.91
Sesame seeds flour (*Sesamum indicum)*	11.20± 0.10	3.30± 0.10	-7.90	20.30± 0.15	25.40± 0.005	5.1	18.12± 0.10	19.20± 0.005	1.08	36.50± 0.50	45.90± 0.10	9.4	2.50± 0.010	3.01±0. 0005	0.51
Pumpkin seeds flour (*Cucurbita maxima)*	10.31± 0.15	0.31± 0.10	-10.00	9.90± 0.06	11.90± 0.001	2.00	25.10± 0.15	30.10± 0.001	5	49.50± 0.15	51.90± 0.15	2.4	4.50± 0.15	5.84± 0.001	1.34

Values are expressed in mean ± SD (Standard Deviation), **Each value is a mean of triplicate determinations with Standard Error

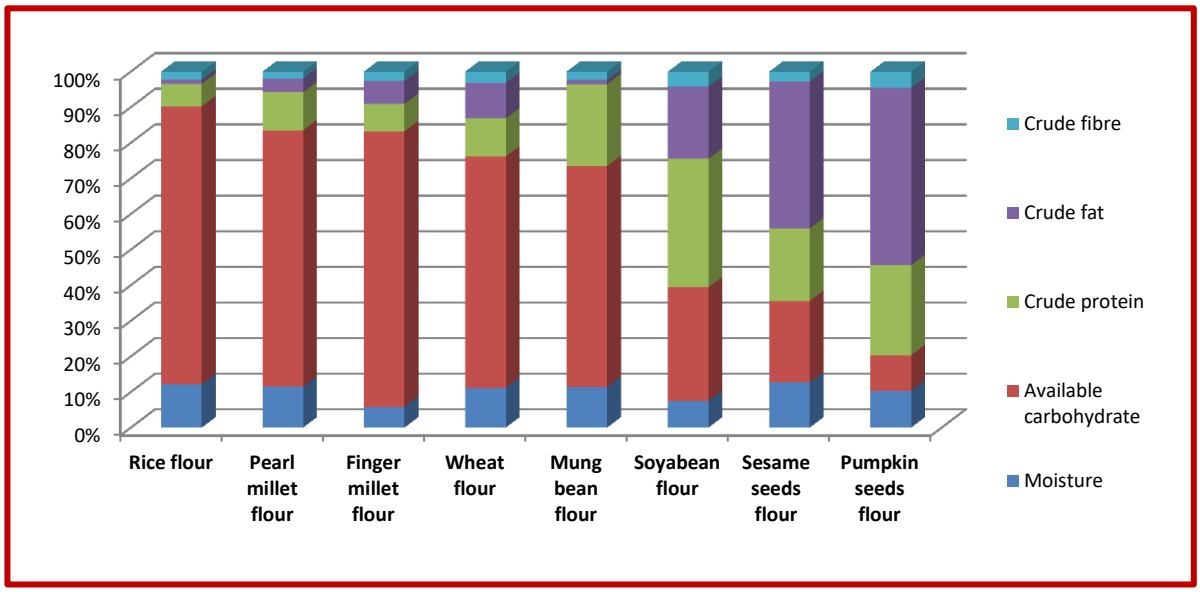


Fig. 4.1. Nutrient composition of raw ingredients before processing

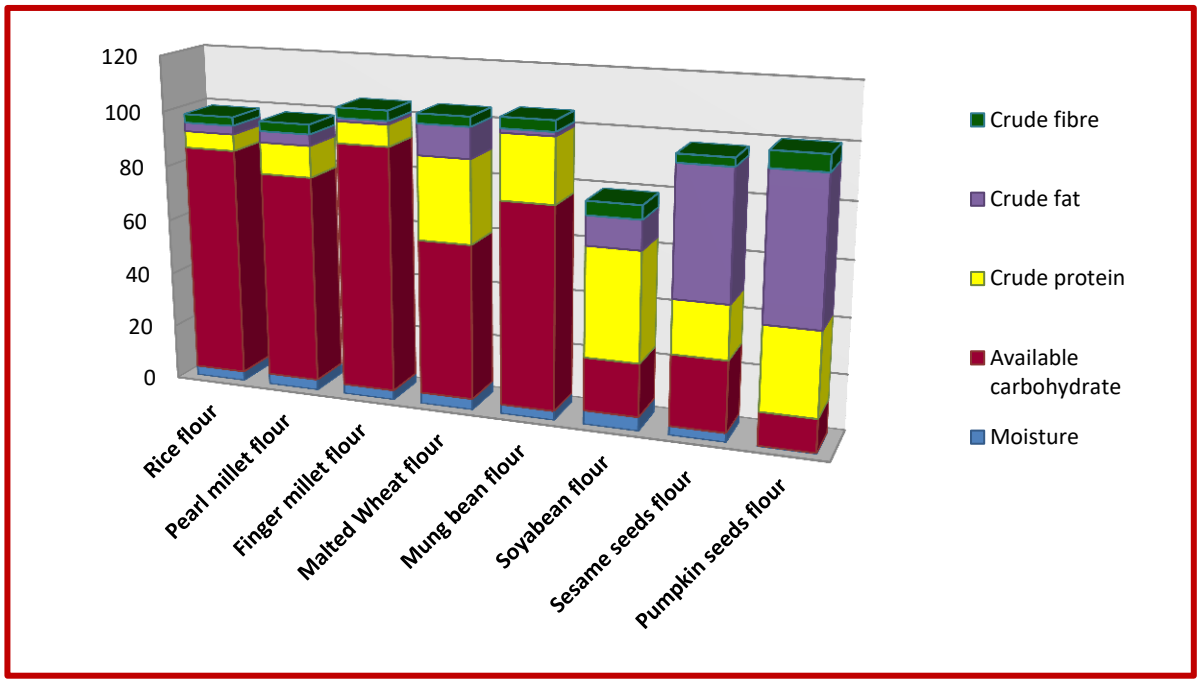


Fig. 4.2. Nutrient composition of raw ingredients after processing

Moisture

The moisture content of rice flour, pearl millet flour, finger millet flour, wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour before primary processing were 11.20±0.01 g/100g, 11.20±0.05 g/100g, 5.58±0.20 g/100g, 11.01±0.15 g/100g, 10.00±0.05 g/100g, 7.05±0.10 g/100g, 11.20±0.10 g/100g, 10.31±0.15 g/100g, respectively.

The moisture content of rice flour, pearl millet flour, finger millet flour, malted wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour after primary processing were found to be 3.46±0.06 g/100g, 3.90±0.10 g/100g, 3.50±0.10 g/100g/100g, 4.01±0.10 g/100g, 3.36±0.10 g/100g, 5.02±0.10 g/100g, 3.30± 0.10 g/100g and 0.31± 0.10g/100g, respectively.

Jung *et al.,* (2018) stated that the total amount of moisture content present in raw ingredients before processing is an important characteristic for determining the physical properties of the developed product. The physical properties of a food material may be negatively impacted by even a slight deviation from a specified requirement. Food samples that are overly dry may influence the final product's consistency. On the other hand, when samples have high moisture content the rate of microbial development increases leading to spoilage of the product (Appoldt and Raihani, 2017). Reduced moisture levels aid in preserving products during storage, extending the product's shelf life and increasing nutrient concentration, which may allow certain nutrients to be more easily absorbed (Morris *et al.,* 2004).

From Table 4.1, it is revealed that there is a significant decrease in the moisture content of the raw ingredients before and after processing. This may be attributed to the different roasting and drying temperatures in which the raw ingredients was subjected to. The high moisture content in soyabean among legumes i.e. 5.02±0.10 g/100g may be a result of the whole seeds absorbing moisture from the soaking media, or water, during the metabolic changes process. The imbibitions of water by dry seed coat increase the moisture content of the seeds. Due to imbibitions of water the seed coat becomes more permeable to water, and moisture retention is more (Chamba *et al*., 2021). Hence in the present

study the raw ingredients were dried to such an extent that the shelf life and the overall acceptability of the product were increased.

Available carbohydrate

It can be inferred from the above data that the increase in the available carbohydrate content before and after the processing as shown in Table 4.1 was due to the standard protocol used for calculating the carbohydrate. The available carbohydrate content of rice flour, pearl millet flour, finger millet flour, wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour before processing were 72.04±0.05 g/100g, 70.00±0.07 g/100g, 74.03±0.05 g/100g, 63.86 ± 0.005g/100g, 54.09±0.05 g/100g, 30.10±0.06 g/100g, 20.30±0.015 g/100g and 9.90± 0.06 g/100g, respectively. The available carbohydrate content of rice flour, pearl millet flour, finger millet flour, malted wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour after processing were 82.94±0.005 g/100g, 75.00±0.010 g/100g, 88.50±0.015 g/100g, 55.86±0.01 g/100g, 73.00±0.005 g/100g, 19.08±0.005 g/100g, 25.40±0.005 g/100g and 11.90± 0.001 g/100g, respectively.

House hold methods such as washing, soaking, drying, roasting, removing the seed coat, and germinating help in the enhancement of the nutritional quality of cereals and legumes. The starch digestibility significantly increases from 36.3 % to 39.2% when the cereals and legumes go through germination and dehulling while processes such as soaking, roasting, and removal of seed coats bring significant changes in the complex carbohydrates (Ghavidel and Prakash, 2007; Egounlety and Aworh, 2003). The processing techniques employed in the present study were in accordance with the results of the above authors.

McAllan *et al.,* (2014) and Brubaker (2011) in their studies revealed how the energy density of a food product is affected by macronutrient constituents such as carbohydrates. They have also revealed that the dietary carbohydrates have a potential affect on enriching the energy content of food mixes. The dietary carbohydrates when digested get metabolized into smaller pieces called glucose which is the major sugar that serves as an energy source for the highly energy demanding cells of the brain, muscles, and nervous system of the human body.

As in the present study the energy density of food products plays a major role hence carbohydrates content of raw materials was conducted.

Crude protein

The crude protein content rice flour, pearl millet flour, finger millet flour, wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour before processing were 5.85±0.01 g/100g, 10.40±0.50 g/100g, 7.42±0.05g/100g, 10.50±0.03 g/100g, 20.02±0.05 g/100g, 34.06±0.10 g/100g, 18.12±0.10 g/100g, and 25.10±0.15 g/100g, respectively. The crude protein content rice flour, pearl millet flour, finger millet flour, malted wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour after processing were 6.00±0.005 g/100g, 11.40±0.005 g/100g, 7.80±0.001g/100g, 30.00±0.005 g/100g, 23.32±0.001 g/100g, 39.28±0.005 g/100g, 19.20±0.005 g/100g, and 30.10±0.001 g/100g, respectively.

Gupta *et al.,* (2013) mentioned that soaking and roasting help in increasing the protein quality of commonly consumed cereals and legumes as much as 23%. Research conducted by Jitender *et al*., (2018) attributed the significance of green gram in the Indian food system due to its easily digestible protein and its valuable properties that contain 24% protein, 1.3% fat, 124 mg calcium, 326 mg phosphorus and 7.3 mg of iron.

Fat

From Table 4.1, it can be inferred that among all the functional ingredients, sesame seeds and pumpkin seeds had the highest amount of fat content. The fat content of rice flour, pearl millet flour, finger millet flour, wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour before processing were 1.13±0.02 g/100g, 3.80±0.14 g/100g, 6.20±0.06 g/100g, 11.05±0.05 g/100g, 1.05±0.001 g/100g, 19.08±0.01 g/100g, 36.50±0.50 g/100g, 49.50± 0.15 g/100g respectively.

The fat content of rice flour, pearl millet flour, finger millet flour, malted wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour after processing were 3.14±0.38 g/100g, 4.08± 0.14 g/100g,

1.20± 0.05 g/100g, 9.80±0.002 g/100g, 1.48±0.25 g/100g, 10.48±0.13 g/100g, 45.90±0.10 g/100g, 51.90±0.15 g/100g respectively.

The lipid content of the soyabean flour increased from 0% to 11%, although this rise was not statistically significant. It has been noted that after roasting, cereal grains, especially millets and lentils, contain more fat. The breakdown of cell structure and effective release of oil reserves may be the causes of the rise in crude fat content as reported by Agume *et al*. (2017).

Similar results were reported by Prasad *et al.,* (2012) and Syed (2019) stating that sesame seeds and pumpkin seeds contain fats in the amount of 50.87 g in 100g and 49.05 g in 100g respectively. The fat content was found maximum in sesame seeds and pumpkin seeds in the present study as quoted by Rolls (2007) who places fat estimation in higher position for increasing the energy density of food.

Crude fibre

The crude fibre content of rice flour, pearl millet flour, finger millet flour, wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour before processing were 2.05±0.01 g/100g, 1.88±0.015 g/100g, 2.50±0.06 g/100g, 3.50±0.05 g/100g, 2.05±0.06 g/100g, 4.00±0.50 g/100g, 2.50±0.010 g/100g, and 4.50±0.15 g/100g respectively.

The crude fibre content of rice flour, pearl millet flour, finger millet flour, malted wheat flour, mung bean flour, soyabean seeds flour, sesame seeds flour, and pumpkin seeds flour after processing were 3.05±0.001 g/100g, 3.49±0.001 g/100g, 3.65±0.005 g/100g, 3.12±0.001 g/100g, 3.91±0.005 g/100g, 4.91±0.001 g/100g, 3.01±0.005 g/100g, and 5.84±0.001 g/100g respectively

Similar results of fibre content were reported by Mushtari *et al.,* in 2017 stating that finger millet contains 3.6 g of protein in 100 g of sample. Out of the pulses, soyabean contained the highest amount of fibre and similar findings were reported by Gupta in 2013 i.e. 4.9±0.18 g/100g. In the present study pumpkin seeds contained highest amount of fibre (5.84±0.001g) after processing among the oil seeds and the results were similar with the findings of Syed *et al.,* in 2019 that stated 6 g of fibre was present in 100 g of pumpkin seeds.

4.1.2. Impact of malting on the nutrient composition of wheat (*Triticum aestivum)* flour.

The impact of malting on the nutrient composition of wheat (*Triticum aestivum)* flour was compared with the raw wheat flour by independent t- test and is presented in Table 4.2.

Table 4.2. Impact of malting on the nutrient composition of wheat (*Triticum aestivum)* flour.

Ingredients	Nutrients (per 100 g of sample in dry weight basis)				
	Moisture (g)	Available carbohydrate (g)	Crude protein (g)	Crude fat (g)	Crude fibre (g)
Raw wheat flour (*Triticum aestivum*)	11.01±0.15	63.86±0.005	10.50±0.03	11.05±0.05	3.50±0.05
Malted wheat flour (*Triticum aestivum*)	4.01±0.10	55.86±0.01	30.00±0.005	9.80±0.002	3.12±0.001
t- test	31.23**	37.16**	25.05**	29.54**	31.46**

Values are expressed in mean ± SD (Standard Deviation)

****** Signifcant at p<0.01, NS= Non significant

The conventional, non-thermal process of germination (sprouting) enhances the nutritional value of cereals and pulses by increasing the digestibility of nutrients, lowering the levels or activities of anti-nutritional components, increasing the contents of free amino acids and available carbohydrate, and enhancing functionality (Rusydi, 2011; Kavitha and Parimalavalli, 2014).

Moisture content significantly reduced (p<0.01) after malting, as the wheat grains were subjected to oven drying after germination. Hence the moisture

content significantly reduced for the malted wheat flour as compared to the raw wheat flour.

Available carbohydrate was significantly lower ($p<0.01$) in the malted wheat flour as the metabolic changes takes place due to the process of germination that uses the stored energy in the form of glucose as compared to the raw and roasted samples. The process of sprouting activates the enzymatic activity in germinated grains resulting in breakdown of complex carbohydrates into simple glucose. Hydrolytic enzymes are activated during germination, as per studies conducted on a variety of cereal grains, and results in the deterioration of starch and non-starch cellulose and an increase in reducing sugars as well as the release of insoluble phenols chemically linked to cell wall polysaccharides (Kavitha and Parimalavalli, 2014).

Crude protein significantly increased ($p<0.01$) in the malted wheat flour as a result of the enzymatic hydrolysis of components such as phytic acid, polyphenols, and protease inhibitors, which improved protein digestibility after germination. Pandhare *et al.,* (2011) also found a substantial improvement in crude protein content of flours made from germinated cereal grains and was attributable to the activation of the hydrolytic enzymes namely gibberellin, which increased the water activity. The endosperm becomes starchy and has more protein because gibberellin stimulates the manufacture of amylase in the aleurone layer

Crude fat content significantly reduced in malted wheat flour ($p<0.01$) when compared to raw wheat flour. In 2014, Chaudhury and Vyas also reported that process of germination was responsible for decrease in crude fat content due to loss of total solids during soaking and probably as well as usage of fat as source of energy during the germination process. During soaking the lipase enzymes break down fats into lycerin and fatty acids and since these compounds are water soluble, they can diffuse into the cells and tissues (Narsih *et al.,* 2012). Many studies also reported that during germination of seeds, the decreasing amount of fat is due to the increased activity of lipolytic enzymes during germination, which

hydrolyze the fats into fatty acid and glycerol (Badshah *et al.*1991; Chung *et al.*1998; Inyang and Zakari, 2008)

Crude fibre content significantly reduced in malted wheat flour ($p<0.01$) when compared to raw wheat flour. The results of the present study was similar to the work done by Laxmi *et al., (*2015) who observed a significant decrease in crude fibre content after sprouting of chickpea due to the fact that during germination, activity of enzyme-ß-galactosidase increases which leads to reduced levels of crude fibre. In 2003, Hooda and Jood reported that during malting the activity of enzyme ß-galactosidase increases which leads to reduced levels of crude fibre during germination.

From the present study, it was observed that germination and malting has a significant impact on the nutrient composition of wheat. Study results also revealed that the nutritional content of malted wheat flour significantly ($p<0.01$) improved compared to the raw wheat flour.

4.1.3. Mineral composition of the raw ingredients used for formulations

The mineral composition of the raw ingredients used for the study is presented in Table 4.3.

Table 4.3. Mineral composition of functional ingredients used for formulation of complementary food mixes

Ingredients	Nutrients (per 100 g of sample in dry weight basis)			
	Calcium (mg)	Iron (mg)	Zinc (mg)	Phosphorus (mg)
Rice flour	9.91±0.005	0.65±0.005	1.31±0.010	159.01±0.010
Pearl millet flour	42.01±0.010	5.91±0.010	2.20±0.005	191.00±0.005
Finger millet flour	330.01±0.01	6.71±0.01	3.00±0.005	299.01±0.010
Malted wheat flour	39.1±0.005	5.02±0.010	2.11±0.010	811.00±0.005
Mung bean flour	121.50±0.20	4.51±0.010	2.91±0.005	311.01±0.010
Soyabean flour	220.01±0.010	11.01±0.005	3.31±0.010	675.01±0.015
Sesame seeds flour	1330.01±0.010	8.91±0.010	12.11±0.010	551.69±0.010
Pumpkin seeds flour	51.01±0.010	3.30±0.011	7.50±0.011	400.01±0.015

*Values are expressed in Mean ± Standard Deviation (SD)

**Each value is a mean of triplicate determinations with Standard Error

Calcium

Calcium (Ca) deficiency is often visually not detectable, and hence is challenging to diagnose in the early stages, but if it is identified it is generally simple to treat by improving dietary Ca intake or absorption. Calcium is a mineral that is essential for healthy bone development in infants. The most effective strategy to prevent calcium deficiency appears to be dietary modifications that include calcium rich foods like dairy products. However, convincing people to adopt diversified diets is difficult, and some food items cannot be added in the meals. For instance, 65% of people worldwide cannot get their calcium needs from dairy products because they are lactose intolerant (Wang *et al.* 2013).

The highest content of calcium among cereals was found in finger millet flour i.e. 330.01±0.01 mg. Devi *et al.,*(2014) and Kumar *et al.,*(2016)found that finger millet contains the highest calcium content among all cereals (344 mg/100 g). When compared to other major cereal grains, such as rice and wheat. Among pulses the highest calcium content was found in soyabean flour 220.01±0.010 mg per 100 g. Gupta (2013) revealed that 12 hours soaked soyabean have 239±0.05 mg of calcium content which was significantly lower than the raw soyabean. Sesame seeds had the highest calcium content i.e. 1330.01±0.010 mg in 100 g among oil seeds. Mohammad *et al.,* in 2011 stated that 1228 mg of calcium is present in the white variety of sesame seeds (Gopalan *et al.* 1982, Weiss, 1983).

Iron

Children with iron deficiency anaemia (IDA) experience negative effects on their physical and brain development (Anitha *et al.,* 2021). The study reported that finger millet flour contains 6.71±0.01 mg of iron. Pragya and Rita, (2012) stated that the best source of calcium and iron is finger millet. Finger millet incorporated composite flours can be used to create a variety of nutrient dense food products that are useful for supplemental feeding programmes for children. Depending on the processing method, such as raw, roasted, and germinated, the iron level of finger millet varied from 3.3 mg to 14.8 mg. Among pulses, soyabean had the

highest iron content i.e. 11.01±0.005 mg per 100 g. Gupta *et al.* in 2013 found out 8.1±0.30 mg of iron in soyabean seeds when soaked for 12 hours in normal water. Among oil seeds sesame seeds flour had the highest content of iron content i.e. 3.30±0.011 mg in 100 g. Prasad *et al.,* 2012 and Tangko *et al.,* in 2020 stated had similar findings stating that in white sesame seeds 14.55 mg/100g of iron is present.

Zinc

Zinc is an important element performing multiple functions in the human body as it is a co-factor for a number of enzymes. Dietary zinc deficiency can lead to growth failure and poor development of gonadal function as well as dwarfism. Among cereals, finger millet flour had the highest zinc content i.e. 3.00±0.005 mg in 100 g. Present findings for zinc are slightly higher than that of result revealed by Hiremath *et al.,* (2018) for finger millet with zinc content of 2.79±0.05 mg in 100 g.

Among pulses, soyabean had the highest zinc content of 3.31±0.010 mg in 100g. The present findings for zinc was slightly lower to the findings of Gopalan *et al.,* in 2016 in the nutritive value of Indian foods stated that soyabean seeds have 3.40 mg of zinc in 100 g. Among the oil seeds, sesame seeds flour had the highest zinc content of 12.11±0.010 mg.

Phosphorus

Phosphorus is an essential element for a baby's teeth and bones to develop properly. Phosphorus along with calcium and vitamin D formulates the structure of healthy bones and teeth in an infant. Equilibrium is maintained between all the three nutrients through phosphorus. A low phosphorus containing cereal can bring serious changes in the growth of the infant.

Among the cereals, phosphorus content was found to be highest in malted wheat flour i.e. 811.00±0.005 mg. The wheat whole flour had 306 mg of phosphorus and the effect of germination of wheat grains on phosphorus increased its content to 8.4%, which was revealed by a study conducted by Azeke *et al.,* in 2011. Among pulses soyabean flour had the highest content of phosphorus i.e. 675.01±0.015 mg in 100 g. As per the findings of Biel *et al.,*

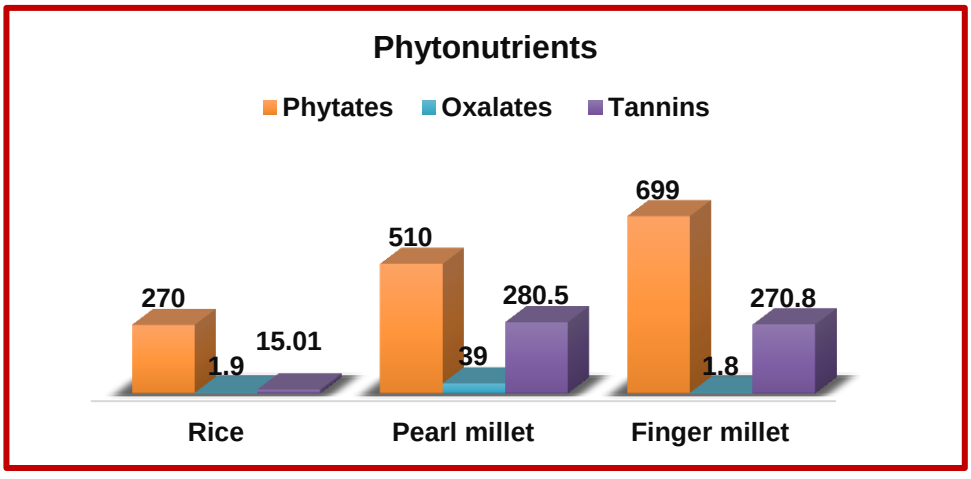
(2018) phosphorus content in two different varieties of finger millet was found to be 10.32 g/kg and 10.37 g/kg namely Aldana and Merlin. Among oil seeds, phosphorus was found to be highest in sesame seeds 551.69±0.010 mg. On the contrary, studies reported by Bamigboye and Adepoju in 2010 stated that 457.0±0.30 mg of phosphorus was present in white whole sesame seeds.

Study results revealed that the sesame seeds flour had the highest calcium content (1330±0.010 mg) and zinc content (12.11±0.010 mg), soyabean flour had the highest iron content (11.01±0.005) and malted wheat flour (811.00±0.005 mg) had the highest phosphorus content.

4.1.4. Phytonutrient content of raw ingredients used for formulation of mixes

Anti-nutritional components decreases the bioavailability of nutrients and also can be toxic if present beyond a permissible limit. Hence, decrease in the amount of these components is an important part of research and also to avoid related health problems associated with these components (Gemede and Ratta, 2014).

In the present study anti-nutritional components like phytates, oxalates and tannins were estimated in cereals and legumes used for formulation of complementary food mixes are presented in Fig. 4.3.



**Each value is a mean of triplicate determinations with Standard Error

Fig. 4.3. Phytonutrient components of functional ingredients used for formulation of complementary food mixes

The results showed that highest phytate content was present in finger millet, among cereals and millets i.e. 699±0.50 mg/100g. Researchers have shown that phytate content in finger millet ranges between 679 mg and 1,419.4 mg per 100 grams depending on different varieties but are higher than rice (289.9 mg/100 g), pearl millet (518.5 mg/100 g), and sorghum (571.1 mg/100 g), but lower than that of wheat (792.1 mg/100 g) (Antony and Chandra, 1999; Makokha *et al.,* 2002; Amalraj and Pius, 2015).

Pearl millet had the highest amount of oxalates among all the cereals and millets i.e. 39 mg/100g (Fig 4.4). The oxalate content of the pearl millet varieties employed in this study were within the range reported in the literature by Lestienne *et al.,* 2005 i.e. 31.6 mg/100 g to 40 mg/100 g.

In the present study tannin content was reported highest in pearl millet i.e. 280±0.10 mg/100g. Millet grains have wide range tannins content (Devi *et al.,* 2014). The range of tannin content in this study was within the value revealed by Amalraj and Pius (2015) stating that pearl millet contain higher tannin content i.e. 275.8 mg/100 g much higher than finger millet 264.1 mg/100 g and rice (14.3 mg/100 g).

PHASE II

4.2 Formulation and standardization of complementary food mixes

For the estimation of energy density in the developed formulations, the carbohydrate, protein and fat content of raw ingredients were determined for each ingredient. A total of six test samples were formulated using different levels of incorporation (Table 4.4). The total energy content of the formulations were determined by adding the estimated value of carbohydrate, protein and fat of all the ingredients for each test samples and then multiplying with a constant factor of 4 for carbohydrate and protein and 9 for fat (ICMR, 2010).

Table 4.4. Determination of energy density of formulated complementary food mixes value per 100 gm for product categorization.

Treatments	Ingredients								Energy Density (ED) KJ/100g
	Cereals/millets (50 %)			Pulses/legumes (25%)		ARF (5 %)	Oil seeds (10% each)		
	R	PM	FM	MB	SB	WF	SS	PS	
T_1	192.01	-	-	-	81.94	23.18	59.15	63.51	419.79 kcal (1756 kJ)
T_2	-	191.16	-	-	81.94	23.18	59.15	63.51	418.94 kcal (1752 kJ)
T_3	-	-	198	-	81.94	23.18	59.15	63.51	425.78 kcal (1781 kJ)
T_4	192.01	-	-	99.65	-	23.18	59.15	63.51	437.50 kcal (1830 kJ)
T_5	-	191.16	-	99.65	-	23.18	59.15	63.51	436.65 kcal (1826 kJ)
T_6	-	-	198	99.65	-	23.18	59.15	63.51	443.49 kcal (1855 kJ)

(R-Rice, PM-Pearl millet, FM-Finger millet, MWF- Malted wheat flour, MB-Mung bean, SB-Soyabean, SS-Sesame seed, PS-Pumpkin seed)

T1 = *Rice* 50% + Soyabean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%

T2 = *Pearl millet* 50% + Soyabean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%

T3 = *Finger millet* 50% + Soyabean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%

T4 = *Rice* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%

T5 = *Pearl millet* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%

T6 = *Finger millet* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%

From the above table it can be inferred that out of the six formulated test samples, T_6 developed using pearl millet flour + mung bean flour + MWF + sesame seeds flour + pumpkin seeds flour had the energy density value of 443.49 kcal/100g sample or 1855 kJ T_4 developed using rice flour + mung bean flour + MWF + sesame seeds flour + pumpkin seeds flour had the energy density value of 437.50 kcal or 1830 kJ after T_6.Two formulations had the highest energy density value among all the six values and hence were subjected to probiotification and further to *In-vitro* and *In-vivo* analyses.

WHO (2001) stated that in developing countries like India the infants who consume average amount of breast milk, the requirement of energy needs from complementary food increases from 200 kcal/day for 6-8 months to 300-550 kcal/day for 9-11 months and 12-23 month old children. As the child grows the intake of breast milk decreases with increase in energy requirement hence

increase in intake of complementary food mixes. The quantity or frequency of meals depends on how much breast milk is consumed along with the age of the children. The older children may require a larger quantity of complementary food mixes than the younger children depending on their energy needs.

PHASE III

4.3. Probiotification of complementary food mixes

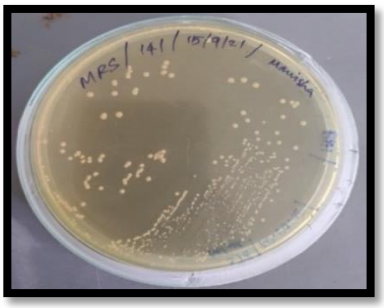
The complementary food mixes were probioticated with *Lactobacillus fermentum* (NDRI-141), *Lactobacillus casei* (NDRI-297), *Lactobacillus rhamnosus* (NDRI-353), and *Lactobacillus plantarum* (NDRI-375) separately.

4.3.1. Morphological and biochemical characterization of bacterial cultures

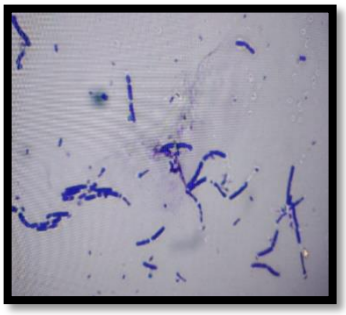
The morphological and biochemical characterization of the micro-organisms are presented in Table 4.5. Plate 22 shows the morphological and biochemical characterisation of all four *Lactobacillus* strains.

Table 4.5. Morphological and biochemical characterization of bacterial cultures

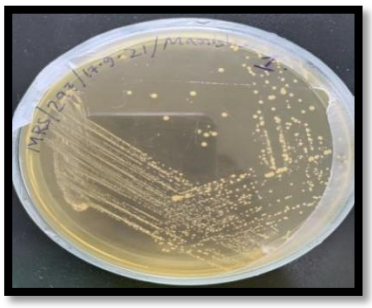
Bacterial Cultures	Morphological characteristics of bacterial cultures			Biochemical characteristics of bacterial cultures
	Type of colony	Colony colour	Gram reaction	Shape and Gram reaction
Lactobacillus fermentum (NDRI-141)	Small	White, shiny	+	Long and slender rods, in chains or palisades
Lactobacillus casei (NDRI-297)	Small	Yellow cream, shiny	+	Square ends, occurring singly, in pairs, or in chains
Lactobacillus rhamnosus (NDRI-353)	Small	Creamy white, shiny	+	Elongated rod shaped and are not assembled in pairs
Lactobacillus plantarum (NDRI-375)	Small	Creamy white, shiny	+	Elongated rod shaped assembled in pairs or in chains



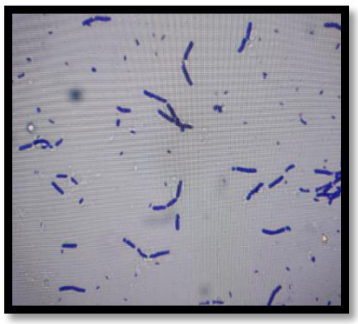
Morphological characterization of *Lactobacillus fermentum*



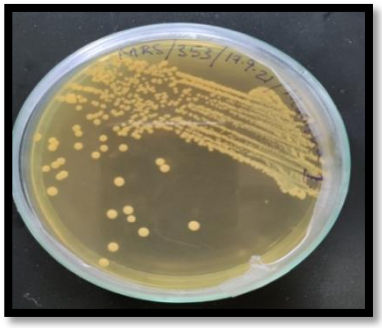
Biochemical characterization of *Lactobacillus fermentum*



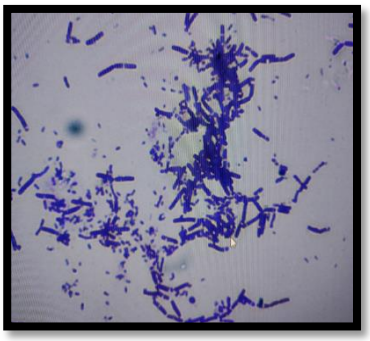
Morphological characterization of *Lactobacillus casei*



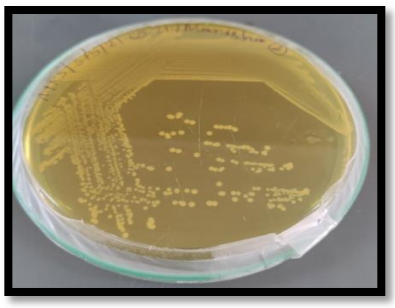
Biochemical characterization of *Lactobacillus casei*



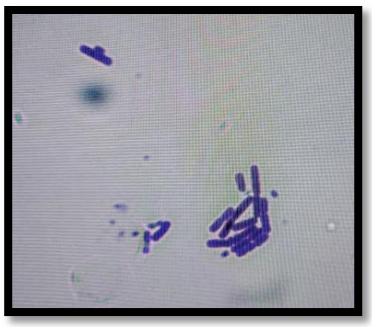
Morphological characterization of *Lactobacillus rhamnosus*



Biochemical characterization of *Lactobacillus rhamnosus*



Morphological characterization of *Lactobacillus plantarum*



Biochemical characterization of *Lactobacillus plantarum*

Plate.22. Morphological and biochemical characterization of *probiotic bacteria*

Morphological characterization of *Lactobacillus fermentum* showed that they were small, white and shiny in colour. Biochemical characterization of *Lactobacillus fermentum* showed that they are gram positive having long and slender rods, in chains or palisades characteristics.

Morphological characterization of *Lactobacillus casei* showed that they were small, yellow and shiny in colour. Biochemical characterization of *Lactobacillus casei* showed that they are gram positive having square ends, occurring singly, in pairs, or in chains characteristics.

Morphological characterization of *Lactobacillus rhamnosus* showed that they were small, creamy and shiny in colour. Biochemical characterization of *Lactobacillus rhamnosus* showed that they are gram positive having elongated rod shape and were not assembled in pairs characteristics.

Morphological characterization of *Lactobacillus plantarum* showed that they were small, white and creamy in colour. Biochemical characterization of *Lactobacillus plantarum* showed that they are gram positive having elongated rod shape assembled in pairs or in chains characteristics.

4.3.2. Microbiological evaluation of test samples before and after freeze drying

The viability of probiotic microbial cultures (*Lactobacillus fermentum, Lactobacillus casei, Lactobacillus rhamnosus and Lactobacillus plantarum*) in test samples (T_4A1, T_4A2, T_4A3, T_4A4) and (T_6B1, T_6B2, T_6B3, and T_6B4) before and after freeze drying are presented in Table 4.6.

Table 4.6. Microbiological evaluation of samples before and after freeze drying

Viable counts Cfu/ml	T_4				T_6			
	T_4A1	**T_4A2**	**T_4A3**	**T_4A4**	**T_6B1**	**T_6B2**	**T_6B3**	**T_6B4**
Before freeze drying	6.2×10^8	7.1×10^8	7.3×10^8	7.9×10^8	6.4×10^8	8.1×10^8	7.6×10^8	7.7×10^8
After freeze drying	5.5×10^8	5.6×10^8	5.3×10^8	6.0×10^8	5.4×10^8	7.2×10^8	6.5×10^8	5.9×10^8

T_4A1- T_4 + *Lactobacillus fermentum,* T_4A2- T_4 + *Lactobacillus casei,*
T_4A3- T_4 + *Lactobacillus rhamnosus,* T_4A4- T_4 + *Lactobacillus plantarum*
T_6B1- T_6 + *Lactobacillus fermentum,* T_6B2- T_6 + *Lactobacillus casei*
T_6B3- T_6 + *Lactobacillus rhamnosus,* T_6B4- T_6 + *Lactobacillus plantarum*

Rice based mix (T_4) with the highest energy density was inoculated with *Lactobacillus fermentum*, *Lactobacillus casei, Lactobacillus rhamnosus,* and *Lactobacillus plantarum respectively.* Four test samples were formulated namely T_4A1 i.e. T_4inoculated with *Lactobacillus fermentum*, T_4A2 i.e. T_4 inoculated with *Lactobacillus casei*, T_4A3 i.e. T_4inoculated with *Lactobacillus rhamnosus* and T_4A4 i.e. T_4inoculated with *Lactobacillus casei.* The test sample having highest cfu count after freeze drying was designated as CFM I.

The initial cell count of T_4A1 inoculated with *Lactobacillus fermentum* was 6.2×10^8 which decreased to 5.5×10^8 after freeze drying. The initial cell count of T_4A2 inoculated with *Lactobacillus casei* was 7.1×10^8 which decreased to 5.6×10^8 after freeze drying. The initial cell count of T_4A3 inoculated with *Lactobacillus rhamnosus*was 7.3×10^8 which decreased to 5.5×10^8 after freeze drying. The initial cell count of T_4A4 inoculated with *L. plantarum* was 7.9×10^8 which decreased to 6.0×10^8 after freeze drying. Among all the four test samples, T_4A4 inoculated with *Lactobacillus plantarum* showed highest cell viability (6.0×10^8 cfu/ml) after freeze drying. Thus, T_4A4 having highest microbial cell count was designated as CFM I (Table 4.6).

Finger millet based mix (T_6) with highest energy density was inoculated with *Lactobacillus fermentum*, *Lactobacillus casei, Lactobacillus rhamnosus,* and *Lactobacillus plantarum respectively.* Four test samples were formulated namely T_6B1 i.e. T_6inoculated with *Lactobacillus fermentum*, T_6B2 i.e. T_6inoculated with *Lactobacillus casei*, T_6B3 i.e. T_6 inoculated with *Lactobacillus rhamnosus* and T6B4 i.e. T_6 inoculated with *Lactobacillus casei*. The test sample having highest cfu count per ml of test sample after freeze drying was designated as CFM II.

The initial cell count of T_6B1 inoculated with *Lactobacillus fermentum* was 6.4×10^8 which decreased to 5.4×10^8 after freeze drying. The initial cell count of T_6B2 inoculated with *Lactobacillus casei* was 8.1×10^8 which decreased to 7.2×10^8 after freeze drying. The initial cell count of T_6B3 inoculated with *Lactobacillus rhamnosus*was 7.6×10^8 which decreased to 6.5×10^8 after freeze drying. The initial cell count of T_6B2 inoculated with *Lactobacillus casei* was 8.1×10^8 which decreased to 7.2×10^8 (Table 4.6) after freeze drying. Among all four test samples, T_6B2 inoculated with *Lactobacillus casei* showed highest cell viability (7.2×10^8

cfu/ml) after freeze drying. Thus, T_6B2 having highest microbial cell count was designated as CFM II (Table 4.6).

Zhang *et al.,* (2020) revealed that the ultra-low temperature should only be applied for the effective and successful method for long term preservation of microbes as such as probiotic bacteria, viruses and functional proteins.Mamun *et al*., (2021) concluded in his study that *L. plantarum* had 95% survival rate during low temperature drying at -40^{o}C.

Rizal *et al., (*2020) studied about the effects of low-temperature of -10°C storage on the viability of *Lactoacillus casei* and found out that for maintaining the viabilityand characteristics of *Lactoacillus casei*, they are usually stored at low temperatures. Erliana *et al.,* (2020) revealed that the pH and low temperature are essential factors, which influence the activity of microorganisms to produce lactic acid. The microorganisms are most active at optimum pH and low temperature.

The present study revealed that drying at -53°C for the developed probiotic complementary food mixes showed high count of viable microorganisms after freeze drying. Hence the medium for drying the fermented mixes was found to be suitable strategy.

4.3.3. Resistance to gastric acidity of probiotic complementary food mixes at different pH values at different exposure time

The effect of pH on the viability of the developed probiotic complementary food mixes are presented in Table 4.7.

Table 4.7. Resistance to gastric acidity of probiotic complementary food mixes at different pH values at different exposure time

Treatments	pH Range	Total number of colonies (cfu/g)	
		3 hours	6 hours
CFM I	2	2.0×10^{8}	1.4×10^{8}
CFM I	2.5	3.9×10^{8}	3.5×10^{8}
CFM I	3	4.5×10^{8}	3.9×10^{8}
CFM I	7	5.0×10^{8}	4.6×10^{8}
CFM II	2	4.5×10^{8}	4.0×10^{8}
CFM II	2.5	5.0×10^{8}	4.4×10^{8}
CFM II	3	5.6×10^{8}	5.5×10^{8}
CFM II	7	6.5×10^{8}	6.1×10^{8}

CFM I - T_4 + *Lactobacillus plantarum,* CFM II - T_6 + *Lactobacillus casei*

The bacteria from probiotic complementary food mixes was revived and was grown at different pH levels to study the resistance of the probiotic strains to the acidic environment in the stomach. To test the growth of probiotic strains in CFM I and CFM II different concentrations of bile salts were used.

From the above table it was revealed that at neutral pH 7 the total number of colonies in CFM I was found to be 5.0×10^8 cfu at 3 hours and 4.6×10^8cfu at 6 hours and CFM II was found to be 6.5×10^8 cfu at 3 hours and 6.1×10^8cfu at 6 hours.When compared to the neutral pH, it was reported that the bacteria's viable count reduced as bile salt concentration increased. The probiotic bacteria must to be able to endure the challenging stomach environment and reach the small intestine.The microorganisms that will be considered as probiotics should be able to withstand digestive system inhibitors such as bile saltsand should have certain specific qualities in order to survive the passage to the gut and exhibit their beneficial traits(Havenaar *et al.,* 1992).The capacity to tolerate adverse gastrointestinal environments, particularly acid and ensuing colonisation in the digestive system is one of their most crucial properties of the probiotic strainspresent in the food (Takahashi, 1993; Tambekar and Bhutada, 2010).

The findings of the present study are in par with the findings of Farhangfar *et al*., (2021) who also reported that *L. plantarum* strains found in Siahmazgi cheese can live in pH ranges between 1.50 and 2.50. Even though pH 2.50 is not the most frequent value for the human stomach, it is said to be particularly selective for the selection of bacteria that may be probiotic and ensures the isolation of strains that are very acid resistant.

The viability shown in the present study of the isolates of *L. plantarum* is comparable to those reported by Angmo *et al*., (2016), who observed that the strains of viable *Lactobacillus* spp.including *L. plantarum*, identified from fermented foods was considerably effected by pH 2.00 while no significantdecrease was found at pH 3.00 in contrast to *L. plantarum* strains isolated from traditional Chinese cheese, which had a survival rate of 31.00–80.00 percent at pH 2.50.The findings of the study were in accordance to the present study.

The *Lactobacillus* strains used in the present study showed the tolerance to gastric acidity juice at different pHranging from 2-7 and at different exposure time 3-6 hours.Hence, both the developed complementary food mixes CFM I and CFM II had the specific qualities to resist the gastric acidity as low as 2.00 at different exposure time.

4.3.4 Bile acid resistance of the probiotic complementary food mixes at different concentrations of bile

The bile acid resistance of the probiotic strains present in the probiotic complementary food mixes CFM I and CFM II are presented in Table 4.8.

Table 4.8. Bile acid resistance of the probiotic complementary food mixes at different concentrations of bile

Treatments	Concentration of oxgall(%)	Total number of colonies (cfu/g)
CFM I	0	TNTC
CFM I	0.3	TNTC
CFM I	0.5	5.5×10^{8}
CFM I	1.0	4.9×10^{8}
CFM II	0	TNTC
CFM II	0.3	TNTC
CFM II	0.5	6.9×10^{8}
CFM II	1.0	6.1×10^{8}

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*
TNTC- Too numerous to count

The physiological concentration of bile salts in the small intestine is between 0.2-1.0 (Havenaar *et al.,* 1992).Bile acid in different concentration was taken in order to calculate the colony forming units. Bile tolerance is one of the most important characteristics for probiotic bacteria since it determines their capacity to sustain for colonisation and enzymatic activities in the small intestine. Although inherent bile tolerance appears to be strain-dependent, lactobacilli may adapt to the presence of bile salts over time (Noriega *et al.,* 2004; Guglielmotti *et al.,* 2007; Burns *et al.,* 2010).

Among the developed formulations,(CFM I and CFM II) inoculated with*L. plantarum* and *L. casei,* it was reported that the strains were viable even at a bile acid concentration of 0.5 and 1.0 percent, allowing both the strains to overcome the effect of bile in the gastrointestinal tract. When the bile acid concentrations where 0.0 and 0.3 percent, the colonies were too numerous to count (TNTC).

The viability of the recent *L. plantarum* and *L. casei* isolates are comparable to the observations made by Chou and Weimer (1999), who found that bile-salt strains can also be extracted when strains are subjected to other difficult conditions like as acid pH where bile adopted strains generally show cross resistances to other strain elements.Tambekar and Bhutada (2010) reported thatthe strains are considered as probiotics when there is gradual increase in the concentration of bile acid leading to the increase in total number of colonies in cfu. According to Sanchez *et al.,*(2012), increasing probiotic bile tolerance may aid in the development of stronger strains with increased resistance to other gastrointestinal conditions that threaten probiotic survival. Overall, the bile response is a complex phenomenon that involves several mechanisms aimed at detoxifying bile and reversing its harmful effects on bacterial structures.

Both the strains *L. plantarum* and *L. casei* present in the developed complementary food mixes had the specific qualities to overcome the bile effect in gastrointestinal tract at 0.0 and 0.03 percent concentration.

4.3.5. Activity of antibiotics on common enteropathogens

The activity of antibiotics on the common enteropathogens is presented in Table 4.9.

Table 4.9. Activity of antibiotics on common enteropathogens

Antibiotic	Zone of inhibition	
	O157:H7 (mm)	EHEC (mm)
Ampicillin	18±0.01	15±0.03
Streptomycin	8±0.04	7±0.01
Ciprofloxacin	12±0.02	9±0.02
Vancomycin	10±0.01	9±0.04
Chloramphenicol	9±0.03	8±0.02

O157:H7- E. coli O157:H7 pathotype, EHEC- Enterohemorrhagic *E. coli*

Around the world, antibiotic resistance is becoming to be a serious public health concern. The production of acetic acids and lactic acids, which decreased the pH of the medium, or the competition for nutrients, or the synthesis of bacteriocin or an antibacterial molecule, might all be contributing factors to the antimicrobial activity of the antibiotics.

The above table showed growth inhibition zone of commonly used antibiotics ampicillin, streptomycin, ciprofloxacin, vancomycin and chloramphenicol against two pathotypes. Obtained results showed that the Ampicillin antibiotic had a significant inhibitory effect on both pathotypes EHEC (15±0.03 mm) and O157:H7 (18±0.01 mm) (Fig. 4.4).

Munita and Arias (2016) reported that antibiotic resistance in bacteria can be either inherent or acquired from other microbes. Resistance is acquired by chromosomal mutation or plasmid-mediated gene transfer from one organism to another. Biological forms of resistance, such as antibiotic inactivation, target alteration, or removal of the antibiotic from the cell through efflux pumps, can also be used by bacterial pathogens.

In the present study, the resistance of the isolates to the various antibiotics is similar to the research by other researchers, including Zeighami *et al.,* (2014) and Pourakbari *et al.,* (2013), who found that the EHEC strain causes 12.1 percent and 17 percent, respectively, of diarrhoeal diseases in children under the age of five. E. coli O157:H7 was discovered in 1% of Turkish patients with diarrhoea by Erdogan *et al.,* (2011).

Walsh(2000) reported that the food products that included pasteurised milk and minced beef contained ampicillin-resistant genes from *Salmonella typhimurium* that had been transferred to *Salmonella agona* and *E. coli* K12.As infections can spread from one individual to others due to antibiotic resistance, this issue is a serious problem for the entire world. Genes for antibiotic resistance can also be passed from bacteria at different levels of the food chain.

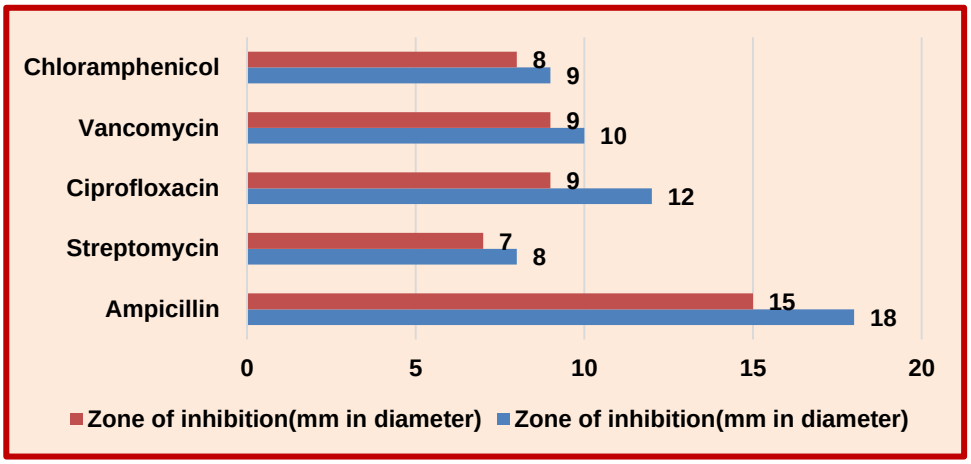


Fig. 4.4. Zone of inhibition (mm in diameter) of antibiotics on common enteropathogens

Out of all the antibiotics used for the study Ampicillin had a significant inhibitory effect on both pathotypes EHEC and O157:H7.

4.3.6. Activity of probiotic complementary food mixes on common enteropathogens

The activity of the probiotic complementary food mix CFM I and CFM II are presented in Table 4.10.

Table 4.10. Activity of probiotic complementary food mixes on common enteropathogens

Probiotics	Pathogens Zone of Inhibition	
	O157:H7(mm)	EHEC(mm)
CFM I	26±0.05	25±0.001
CFM II	28±0.01	26±0.05

O157:H7- E. coli O157:H7 pathotype, EHEC - Enterohemorrhagic *E. coli*
CFM I - T_4 + *Lactobacillus plantarum,* CFM II - T_6 + *Lactobacillus casei*

Probiotic strains had been proven in several studies to suppress the growth of variety of infections. For the common gram negative and gram positive bacteria found in the foods, lactobacillus antibacterial activity was identified.

The table showed growth inhibition zone of probiotic complementary food mixes against two pathotypes O157:H7 and EHEC. Obtained results showed that CFM II had a significant inhibitory effect on both pathotypes O157:H7 (28±0.01 mm) and EHEC (26±0.05 mm). Similarly CFM I also showed inhibitory effect on both pathotypes O157:H7 (26±0.05 mm) and EHEC (25±0.001 mm) but lower than CFM I (Fig. 4.5).

Kargar *et al.,* (2009) revealed that eating yoghurt promotes intestinal colonisation of probiotic bacteria like Lactobacillus and helps in reducing EHEC colonisation. A variety of pathogens, including *Listeria monocytogenes, Salmonella, Shigella, and Staphylococcus aureus*, were shown to be inhibited in growth by various lactobacillus culture supernatants.

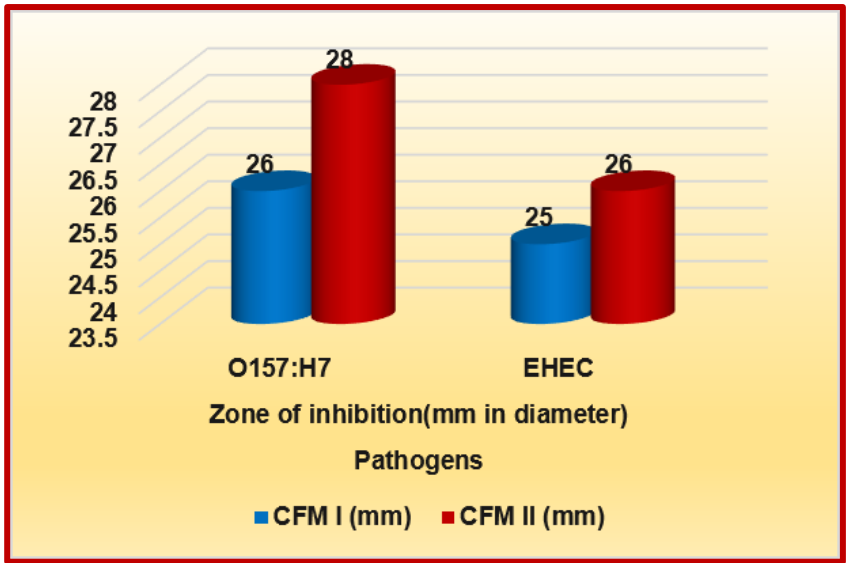


Fig.4.5.Zone of inhibition (mm in diameter) of probiotic complementary food mixes against common pathogens

Lactobacillus casei and Lactobacillus plantarum have been reported by Hassanzadazar *et al*., (2014) to prevent the development of *Salmonella enteritidis*, *Escherichia coli*, and *Listeria monocytogenesis*. Similar investigations supported

the antibacterial properties of probiotic culture supernatants by Mashak in 2016 by demonstrating the growth inhibitory properties of *Lactobacillus curvatus and Lactobacillus plantarum* against a variety of pathogens using the well diffusion method which is also in accordance with the present study.

Several researches have demonstrated that administering probiotics to children can shorten the length of their diarrhoea. Children who have viral diarrhoea brought on by the Rotavirus can be treated with probiotics, which also stops the body from losing water and electrolytes (Allen *et al.,* 2010). Davoodabadi and colleagues (2015) reported on the antimicrobial activity of *Lactobacillus* strains against five pathotypes of *E. coli* that cause diarrhoea and discovered that *Lactobacillus* strains with human origin exhibited a weak inhibitory effect. Suvarna and Boby (2005) reported that irradiation typically causes side effects like abdominal diarrhoea and other gastrointestinal problems. Probiotic product consumption significantly contributes to the avoidance of these issues prior to surgery.

According to the study's findings, probiotic bacteria present in CFM I and CFM IIinoculated with *L.plantarum* and *L.casei* may be employed to suppress and reduce pathogens, particularly enteric pathogens, and antibacterial activities which are active and durable under a range of temperature and acidic circumstances. Plate 23, Plate 24 and Plate 25 shows the zone of inhibition of the antibiotics.

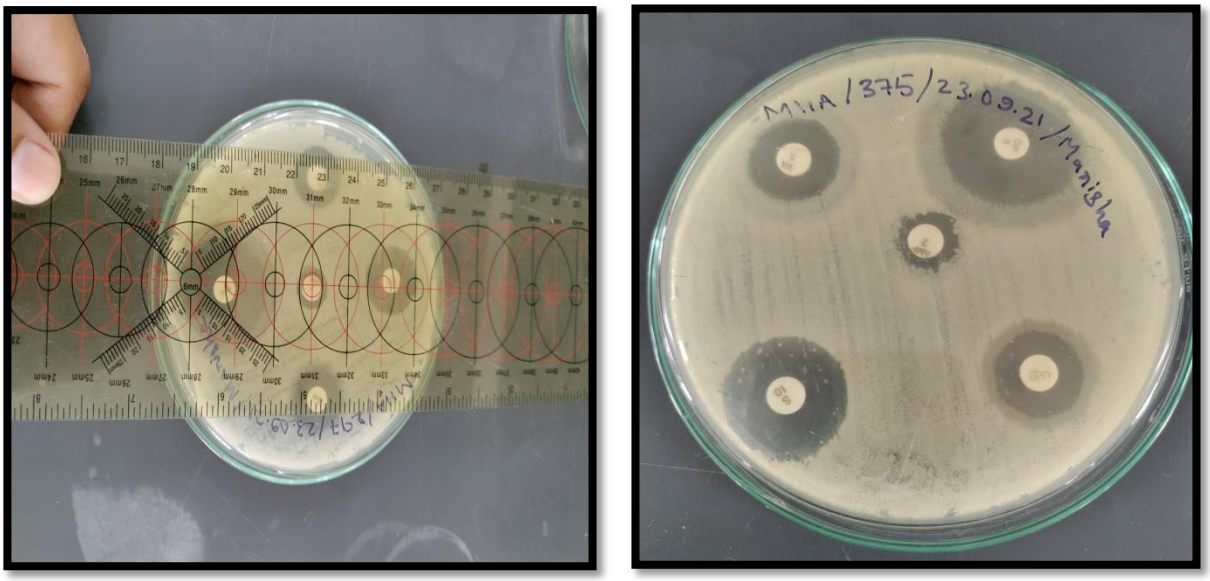


Plate 23. Measurement of the inhibition zone diameter to determine antibiotic susceptibility against probiotic strains

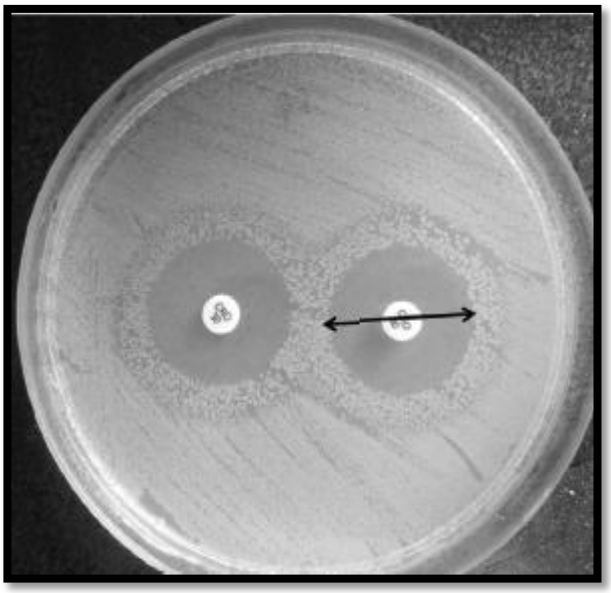


Plate 24. Measurement of the inhibition zone diameter to determine antibiotic susceptibility against *E.coli*

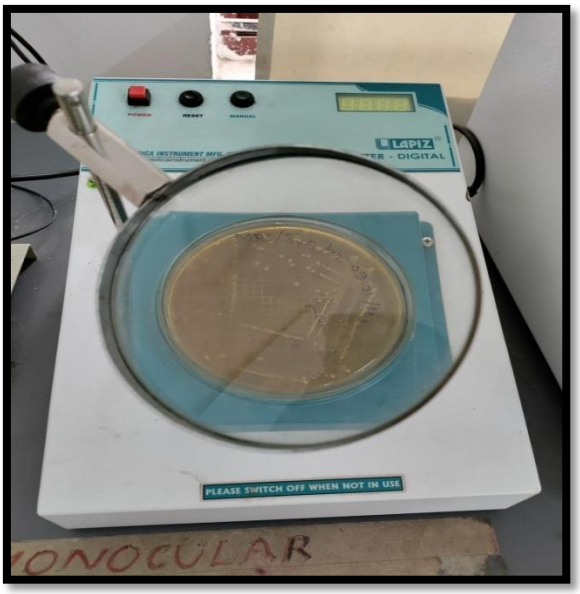


Plate 25. Colony counter

PHASE IV

4.4. Physicochemical potentials of probiotic complementary food mixes

4.4.1 Physical characteristics of developed probiotic complementary food mixes

Table 4.11 gives the physical characteristics of the developed probiotic complementary food mixes.

Table 4.11. Physical characteristics of the probiotic complementary food mixes

Physical parameters	T_4	T_6	CFM I	CFM II
Bulk density (g/ml)	1.03^{b}±0.02	1.48^{c}±0.02	0.54^{a}±0.005	0.52^{a}±0.01
Sedimentation volume (ml)	8.12^{c}±0.02	9.26^{d}±0.20	5.80^{b}±0.01	5.11^{a}±0.01
Swelling Capacity (%)	10.11^{a}±0.01	13.33^{b}±0.57	17.43^{c}±0.01	19.00^{d}±0.10
Water holding capacity (%)	220^{d}±1.00	212^{c}±1.00	200^{b}±1.00	191^{a}±1.00
Fat holding capacity (%)	237.00^{d}±1.00	219.66^{c}±1.52	200.66^{a}±0.57	211.00^{b}±1.00
Dispersibility (%)	42.66^{b}±0.57	41.00^{a}±1.00	64.66^{d}±1.15	57.33^{c}±0.57

Values are expressed in mean ± SD (Standard Deviation), ****** Significant at p ≤ 0.01

Means within rows separated by Duncan's multiple range test p = 0.01

Means followed by the same letter shown in superscript(s) are not significantly different.

T_4 = *Rice* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%, T_6 = *Finger millet* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%, CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

Bulk density

Compared to family foods, complementary food mixes need to be far more nutrient-rich.Low bulk density is a favorable property for complementary food mixes, as porridges made from these powders has lower dietary bulk. High bulk density limits the caloric and nutrient intake in infants and also important in packaging requirement.

It is observed from Table 4.11 that the bulk density of the developed probiotic complementary food mixes ranged from 0.52±0.01 g/ml in CFM I to 0.54±0.005 g/ml in CFM II and were not statistically different at (p≤0.01) from each other whereas the bulk density of untreated complementary food mixes ranged

from 1.03±0.02 g/ml in T_4 to 1.48±0.02 g/ml in T_6 and were statistically different at ($p \leq 0.05$) from each other. The results also showed that the bulk density of CFM I and CFM II was significantly different ($p<0.01$) when compared to the untreated ones.

In 2000, Gokavi and Malleshi stated that fermentation of cereals and legumes elaborates the activity of amylases which in turn is responsible for lowering the bulk density of fermented foods which results in reduced water absorption capacity of flours which is a desirable characteristic for making thinner gruels. Hence more water/milk can be added to the gruel to be prepared as nutrient and energy dense food. Ikujenlola (2008) also reported bulk density of 0.50 to 0.75 g/ml of complementary foods made from germinated and ungerminated soybean along with sesame seeds which were similar to the present study. Other researchers had reported similar findings of bulk density steadily decreasing throughout the fermentation process which might be advantageous for making baby food (Adebowale and Maliki (2011); Oti and Akobundu (2008).On the contrary, studies reported by Nabag (1992) on complementary foods made from legume and carrot reported bulk density (0.64 - 0.80 g/ml) which is higher than the present study that may have been due to other preparation and processing methods of developed samples.

In 2017, Codex Alimentarius confirmed that the predigestion of the starchy element of the meal (dextrinization) is the consequence of the action of microbial organisms, which reduces the bulk of the food and eventually increases the nutritional density of the food. As a result, fermentation has been cited as a practical and age-old technique for making low-bulk supplementary meals (Desikachar,2000; Bala *et al.,* 2014; Ojha *et al.,* 2017; Omobolanle *et al.,* 2015).

Results indicated that probiotificated complementary food mixes are suitable for infants as the samples bulk density values were within the reference range mentioned above.

Sedimentation volume

Sedimentation volume indicates the extent of gelatinization in the material. In the presence of water, gelatinization occurs when the intermolecular

connections between starch molecules are broken down to develop bonds.The results of the present study showed that the sedimentation volume of both CFM I (5.80±0.01 ml) and CFM II (5.11±0.01 ml) significantly decreasedafter probiotificationwhen compared with T4 and T6 were 8.12±0.02 ml and 9.26±0.20 ml respectively at ($p<0.01$) significant level. Similar results of decreased sedimentation volume in pulse powders developed from cow pea, lentil and faba bean was reported by Prabhu *et al.,* (2014). They found that the decreased value of pulse powders after probiotification was due to gelatinization of food particles during fermentation with lactic acid bacteria which were in accordance with the present study.

Swelling capacity

Swelling capacity or swelling power is an indication of the water absorption index of the flour granules during heating. One of the components of starch is the amylose group which has a significant impact on swelling capacity. The lowering of amylase may be due to the extended fermentation process increasing the swelling capacity.

The results of the present study revealed that the swelling capacity of probiotic complementary food mixes CFM I (17.43±0.01) and CFM II (19.00±0.10) significantly ($p\leq0.01$) increased after inoculation of probiotic strains when compared with T_4 and T_6that was 10.11±0.01 % and 13.33±0.57% respectively (Table 4.11).

Several researchers had revealed that a higher protein concentration in flour may result in the starch granules being covered in a rigid protein matrix, limiting the starch's access to water and increasing the swelling potential. The amylopectin is mainly accountable for granule swelling, composite flour's mixes potential to swell would improve if it had more amylopectin and more starch (Loos *et al.,* 1981; Tester and Morrison, 1990; Woolfe, 1992; Aprianita *et al.,* 2009; Sasaki and Matusuki, 2008).According to Moorthy and Ramanujam (1986), the swelling capacity of granules is a sign of how strong the associative forces are inside the granule. Similar results of increased swelling capacity were also reported by Rahmana (2017) on modified fermented breadfruit flour using *Lactobacillus plantarum*which was as par the findings of the present study.

Water holding capacity

A protein matrix's capacity to absorb hydrodynamic, capillary, and physically trapped water against gravity is known as water holding capacity (WHC) and is an essential protein-water interaction that takes place in a variety of food systems.

The water holding capacity of CFM I and CFM II were 200±1.00% and 191±1.00% and T_4 and T_6 were 220±1.00% and 212±1.00%, respectively. It can be observed from the present study that the water holding capacity of probiotic complementary food mixes was significantly lower($p \leq 0.01$) than that of untreated ones (Table 4.11).

Protein-water interactions are related to WHC (Heywood *et al.,*2012; Kinsella, 2016). Therefore, the increase in the amount of protein content after fermentation in probiotic complementary food mixes may influence the WHC values. The enhanced bonding of the structure to absorb and hold water after fermentation leads to, improved taste retention, enhanced tongue feel, and less moisture loss from the developed food products (Prinyawiwatkul *et al.,* 1997; Doke and Duha, 2016).

It was also proven that fermentation increases WHC (Damodaran and Paraf, 1997; Dhingra and Jood, 2004; Traynham*et al.,* 2007). According to previous reports of Desikarchar, (1980), (Ikujenlola, 2004) and Ikujenlola and Adurotoye (2014), the fermentation process causes cereals' natural amylase enzymes to become active. These enzymes dextrinize and saccharify the starch in grains to produce dextrin and maltose, which require minimum water to be cooked.Igbabul *et al.,* (2014) and Msheliza *et al.,* (2018) reported that the WHC of Mahgany bean (*Afzelia africana*) flour dropped during fermentation and freeze drying. Both the mixes CFM I and CFM II were subjected to fermentation and freeze drying resulting in decreased water holding capacity when compared with the untreated ones.

Fat holding capacity

One of the important factors to be considered while formulating food products is the fat holding capacity which helps in improving the flavour and

mouthfeel of foods (Kinsella, 2006). It has been reported that high fat holding capacity is important for increasing energy density of complementary foods. It could therefore be concluded that malted cereals added to complementary foods has a high oil absorption capacity will increase the energy density of the formulated complementary foods.

The fat holding capacity of CFM I and CFM II were 200.66±0.57% and 211.00±1.00% and T4 and T6 were 237.00±1.00% and 219.66±1.52% respectively. It can be observed from the present study that the fat holding capacity of probiotic complementary food mixes was significantly lower ($p \leq 0.01$) less than that of untreated ones

Dispersibility

The dispersibility values of CFM I and CFM II were 64.66±1.15% and 57.33±0.57% and T4 and T6 were 42.66±0.57% and 41.00±1.00% respectively. It can be observed from the present study that the dispersibility of probiotic complementary food mixes was significantly higher ($p \leq 0.01$) than that of untreated ones (Table 4.11).

Ezeocha and Onwuka (2010) revealed in their research on complementary foods made from cereals and legumes, that germination and roasting had a significant impact on the complementary food's dispersibility parameter. The above findings were supported by Akinsola *et al.* in 2017 stating that dispersibility of fermented maize-millet soyabean (68.75±1.37) based complementary food were higher than the control sample (65.75±1.40) and the germinated sample (63.50±0.21).Results from this study indicate that both the mixes were suitable for the preparation of complementary foods.

4.4.2. Viscosity profile of the probiotic complementary food mixes

The viscosity profile of Probiotic complementary food mixes and commercial infant formula at various shear rates are given in Table 4.12.

Table 4.12. Viscosity profile of the probiotic complementary food mixes

Treatments	Viscosity (in centipoises)							
	0.3 rpm	**0.6 rpm**	**1.5 rpm**	**3 rpm**	**6 rpm**	**12 rpm**	**30 rpm**	**60 rpm**
Standard	4591.3^{a} ± 1.52	4756.7^{c} ± 1.70	3570.8^{a} ± 1.9	3470.4^{a}± 1.10	2824.0^{a} ± 3.17	2752.6^{c} ± 1.34	1892.3^{c} ± 3.95	1517.1^{c} ± 0.7
CFM I	4667.8^{b} ±2.25	4570.5^{a} ±3.62	3655.7^{b} ±2.01	3553.2^{b}± 1.12	2966.3^{b} ±0.80	960.4^{b} ±1.15	662.8^{b} ±1.6	446.0^{b}± 1.00
CFM II	4701.4^{c} ±1.01	4672.7^{b}± 2.66	3746.0^{c}± 2.00	3641.2^{c}± 1.08	3959.0^{c} ±3.50	951.0 ±1.00	566.1^{a}± 1.04	401.2^{a}± 1.08

Values are expressed in mean ± SD (Standard Deviation), ****** Significant at $p \leq 0.05$

Means within columns separated by Duncan's multiple range test P = 0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

The flow behavior of probiotic complementary food mixes was observed by measuring the viscosity at different rotational speeds and compared with a commercial infant food formula. Analysis indicated that the viscosity was significantly different ($p<0.05$) at various rotational speeds. All the three mixes exhibited non-Newtonian shear thinning behavior. The viscosity values ranged for CFM I from 4667.8±2.25 cP to 446.0±1.00cP at rotational speeds ranging from 0.3 to 60 rpm and for CFM II from 4701.4±1.01 cP to 401.2±1.08 cP. Viscosities of both the mixes CFM I and CFM II at a rotational speed of 60 rpm were lower, when compared to that of the commercial complementary food formula (4591.3 ± 1.52 cP to 1517.1 ± 0.7 cP) at the same rotational speed (Table 4.12). Reduced viscosity of both the mixes makes them appropriate for feeding babies at an age group of 6-24 months.

Low viscosity value indicates the appropriateness of complementary food mixes for feeding infants. Reduced viscosity is a good indicator of increased nutrient density (Nkama *et al.,* 2001).The high viscosity values of the complementary food mixes at very slow rotational speeds may be due to the differences in dispersion, concentration, and hydration property of various ingredients. Various other factors can also influence the viscosity like pH,

temperature, shear rate, heat treatments and addition of thickeners during the manufacturing process (McCarthy and Singh, 2009; Cichero and Nicholson 2013).Reports by Kinsella and Morr(1984) and McCarthy and Singh(2009) stated that infant formulas exhibit pseudoplastic flow behavior. Fluids exhibit shear thinning over a wide range of shear rates which is a characteristic of fluid milk and protein dispersions containing casein, whey protein, soy protein, etc.This behavior was observed in the present study.The high viscosity values at lower rpm of the complementary food mixes are due to the presence of protein and starch. Starch absorbs water on cooking forming a gelatinous mass. Slight variations in the values are due to the differences in the proportions of each ingredient (Ariahu *et al.,* 1999).

According to research presented by Faki (2004) and Ikujenlola and Fashakin (2005), for all similar slurry concentrations, blends including amylase-rich foods had viscosities that were considerably lower ($p \leq 0.05$) than those of other blends. The amylases that formed during fermentation are responsible for the starch saccharification or dextrinisation that results in the lowering of viscosity in diets made from amylase-rich flours.Helland *et al.,* (2002) stated that during malting amylase activity get increases and alpha amylases degrades starch granules and thereby reduces their water binding capacity, which consequently leads to an reduction in viscosity.Fermentation increases the amylase activity and degrades the starch granule which reduces the water binding capacity of gruels thereby reducing the viscosity. Nout (2004) discovered that *L. plantarum* had an impact on fermented maize samples that increased viscosity regardless of the pH. Researchers had also reported that when malting and fermentation are combined together they are known to be better than malting alone because this combination decreases the viscosity, and also enhances the colour and flavour along with increase in storage quality (Enwere, 1998; Limpisut and Jindal, 2002). From the above studies, it can be concluded that both the developed mixes CFM I and CFM II had high viscosity values when compared with the commercial formula at very high rotational speeds of 60 rpm.A low viscosity value with high nutrient content is a desirable characteristic of complementary foods.

4.4.3. Texture profile of probiotic complementary food mixes

The texture profile of probiotic complementary food mixes and commercial infant formula are given in Table 4.13.

Table 4.13. Texture profile of probiotic complementary food mixes

Group	Adhesiveness (J)	Stickiness (g)	Stringiness (mm)	Resilience
Standard	1.06^{b}±0.03	-3.36^{c}±0.57	0.92^{b}±0.01	0.35^{b}±0.01
CFM I	0.21^{a}±0.03	-4.35^{b}±0.57	0.11^{a}±0.03	0.14^{a}±0.05
CFM II	4.16^{c}±0.03	-5.63^{a}±0.06	3.30^{c}± 0.04	0.10^{a}± 0.04

Values are expressed in mean ± SD (Standard Deviation), ****** Significant at p≤0.05

Means within columns separated by Duncan's multiple range test p=0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I - T_4 + *Lactobacillus plantarum,* CFM II - T_6 + *Lactobacillus casei.*

When it comes to young children's food acceptance, texture is important, especially during the complementary feeding stage.Evaluation of texture is an important criterion for assessing the overall quality of food products. Several instrumental analyses were used for measuring the textural parameters. Based on the texture profile analysis significant difference in adhesiveness and stringiness of complementary food mixes and commercial infant formula can be observed.

Adhesiveness of the CFM I and CFM II probiotic complementary food mixes ranged from 0.21±0.03 J to 1.16±0.03 J. Stickiness varied from -4.35±0.57 to -5.63±0.06 g. Stringiness varied between 0.11±0.03 to 2.30±0.04 mm and resilience ranged from 0.14±0.05 to 0.10±0.04 mm. The textural parameters like adhesiveness stickiness stringiness and resilience values of complementary food mixes were significantly different (p≤0.05) from the commercial complementary food formula.

The textural properties of the complementary foods were influenced by the properties of ingredients. It has been reported that stringy, gummy and slimy foods are rejected (Szczesniak, 2002) and the developmental stages of a child influence what texture are accepted or rejected (Szczesniak, 1972; Lundy *et al.,*

1998). The texture profile analysis of CFM I and CFM II indicated that the parameters measured are convenient for six to twelve month old infants to consume without difficulties.

4.4.4. Proximate composition of the probiotic complementary food mixes

The proximate composition of the developed probiotic complementary food mixes in comparison with the untreated samples is presented in Table 4.14.

Table 4.14. Proximate composition of the probiotic complementary food mixes

Nutrients	T_4	T_6	CFM I	CFM II
Moisture (g)	4.89±0.01^{a}	5.01±0.01^{b}	3.24±0.01^{c}	3.85±0.01^{c}
Available carbohydrates (g)	82.90±0.10^{d}	75.00±0.05^{c,d}	80.24±0.66^{a}	72.83±0.15b
Crude protein (g)	6.90±0.01^{a}	9±0.05^{a}	12.88±0.02^{c}	14.90±0.09^{b}
Crude fat (g)	6.90±0.05^{a}	7.00±0.12^{d}	5.84±0.02^{b}	6.60±0.05^{c}
Crude Fibre (g)	10.00±0.02^{c}	11.59±0.10^{a}	5.62±0.02^{b}	6.12±0.07^{d}
Total minerals (g)	2.50±0.10^{b}	4.00±0.05^{b}	1.23±0.01^{a}	2.00±0.05^{c}

Values are expressed in mean ± SD (Standard Deviation), ****** Significant at p≤0.01

Means followed by the same letter shown in superscript(s) are not significantly different

NS- Not significant, CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

Moisture

The moisture content of CFM I was 3.24±0.01g per 100 g and CFM II developed was 3.85±0.01 g per 100g of sample and were not statistically different at (p≤0.01) from each other, but were statistically different from the untreated ones (Table 4.14).

According to Gopalan *et al.,* (2011), the usage of components such as sesame seeds and rice flour may be the root cause of the increased moisture content of the formulations of complementary mixes.Ukey *et al.,* (2014) formulated complementary mix made from different blends of sun dried barley flour and soy flour with drumstick leaf powder and reported high moisture content of 9.42, 9.84

and 9.85 g/100g However, studies reported by Ugwuona *et al*., (2012) on complementary mixes comprising of varying proportions of soybean seeds and acha grains (*Digitaria exilis*) had lower moisture content ranging from 2.25±0.04 to 3.99±0.61 g/100g which may be due to storing the flours in polyethylene bags and then in airtight containers until needed.

Contrary findings were reported by Ojinnaka *et al*., (2013) on complementary food gruels formulated from blends of soybean flour, ginger, and modified cocoyam starch containing moisture content ranging from 11.55±0.20 to 16.51±0.03 g/100 g.Simwaka *et al.,*(2017) reported significant increase (P≤0.01) in moisture content of a probioticated complementary food mix developed using millet, sorghum, pumpkin and amaranth seeds and stated that the increase in moisture content after probiotification was due to the availability of water in the fermentation medium, the high rate of diffusion of the water molecules from fermenting medium (higher concentration) to food (lower concentration) through osmosis. Similar results of osmotic influence on moisture content were also observed by Hioe*et al.,* 2016 in fermented chickpea and fababean flour. The increase in moisture content was also due to autolysis action of Lactic acid bacteria at this stage of germination, where cell wall collapses with the release of compounds, especially of intact and still active hydrolytic enzymes like esterases, lipase and proteases (Reddy *et al.,* 2009; Morris *et al.,* 2004; Luo *et al.,*2014; Khatoon and Prakash, 2006; Rusydi et al., 2011; Kavitha and Parimalavalli, 2014).

It was evident from the presented data that the developed Probiotic Complementary food mixes in the present study had moisture content below 4 g per 100g of sample, which were within the permissible limit recommended by Bureau of Indian Standards (BIS, 2006) Codex International Standard recommended a maximum level of 10% of moisture for such mixes (CAC, 1994).

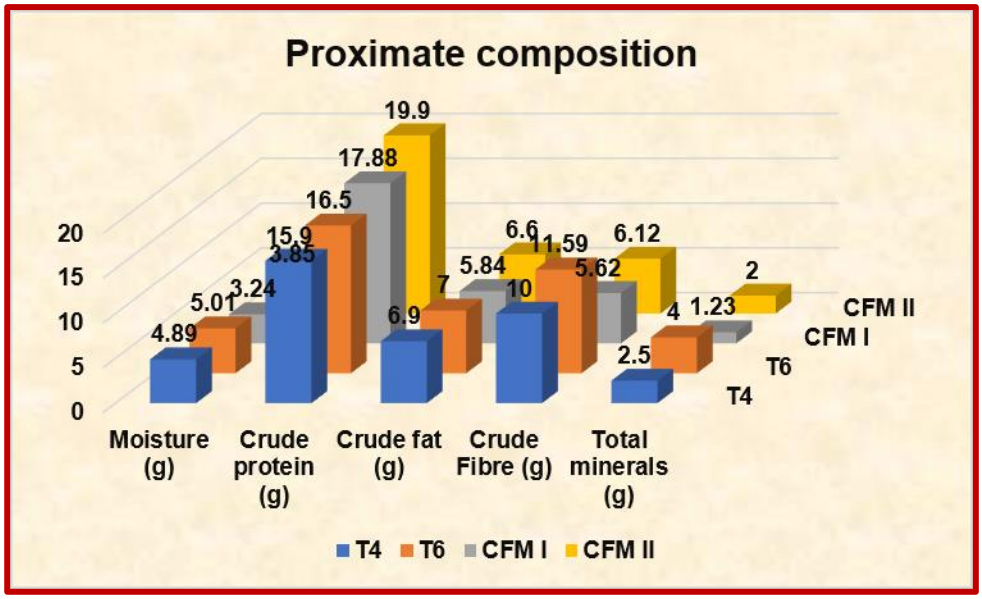


Fig.4.6.Proximate composition of the probiotic complementary food mixes

Available carbohydrates

The available carbohydrates content of CFM I and CFM II were 80.24±0.66g and 72.83±0.15 g per 100g of sample respectively. Fig 4.6.shows that there wassignificant decrease (P≤0.01) in available carbohydrate content of CFM I and CFM II as compared to T_4 and T_6.

The results of the present study were similar to the reported values of carbohydrates ranging from 61.99±0.04 g/100g to 88.15±0.01 g/100g by Nnam (2000) who had developed a complementary food made from soybeans, cowpeas, maize, sorghum, water yams, cocoyams, sweet potatoes and plantains. Ikujenlola and Adurotoye (2014) reported a carbohydrate content ranging from 63.50 to 84.42 g/100g in complementary foods prepared from mixtures of Quality Protein Maize and steamed cowpea which were also similar to the present study. However, contrary studies reported by Achidi *et al.,* (2016) showed lower carbohydrate content (24.56±1.28 to 56.64±3.45 g/100g) when compared to the present study in complementary diets made from rice, soybean, crayfish, carrot, irish potatoes and sugar. This may be due to the use of soybean and irish potatoes in greater proportion in the formulations as compared to rice because the carbohydrate content of soybean (20.9 g/100g) and irish potatoes(22.6 g/100g) are lower than rice (72.5 to 84.3 g/100g).

In 2011, Osman observed decrease in carbohydrate content of a traditional fermented mix called Lohoh developed from pearl millet in Saudi Arabia. He found that decrease in carbohydrate content was probably due to utilization of stored carbohydrates by the active microorganisms during their cell multiplication and proliferation. They stated that decrease in glucose content tend to decrease the carbohydrate content in fermented food products (Khetarpaul and Chauhan, 1990; Simwaka, 2017).

According to BIS (2006), the carbohydrate content should be minimum of 55 g/100g. The codex specified a minimum standard level of 58% carbohydrates.Hence, it can be inferred that all the four probiotic complementary food mixes met with the recommended carbohydrate levels.

Crude Protein

Table 4.14 revealed that the crude protein content of probiotic complementary food mixes of CFM I and CFM II were 12.88±0.02 g and 14.90±0.09 g. Protein content of probiotic complementary food mixes was significantly higher (P≤0.01) than untreated ones.

Similar observations were also made by Desalegn (2015) in a complementary food prepared from blends of Quality Protein Maize and chickpea who reported protein content ranging from 10.42±0.09 to 15.70±0.74%. However, studies done by Akinola *et al.,* (2014) in infant's foods made from soybean and millet in different proportions had higher protein content of 18.15, 16.64 and 21.46% which may be due to the use of high protein sources like soybean and crayfish. The difference in the protein content in these formulations can be attributed to the use of soybean and millet in different proportions.

In 2016, Ambani found significant increased (p≤0.01) protein content from 18.06g to 20.03g per 100 g of lactic acid inoculated fermented cow pea based food mix for the development of value added products. He also reported that the elevated level of protein content was due to the utilization of carbohydrate content needed for the synthesis of proteins for the growth and development of the microorganisms during fermentation which indirectly inhibits the utilization of in-situ protein content in food. In 2009, Food Safety and Standards Authority of India

(FSSAI) also stated that 'Food products nutritional value, especially their protein content, is improved through fermentation'.

Many researchers noted that the increase in crude protein content following fermentation was due to the active proliferation of living microorganisms within the food, which they accomplished by secreting specific extracellular enzymes like amylases, linamarases, and cellulases during their breakdown in the fermentation medium(Ezekiel *et al.,* 2009; Chika *et al.,* 2013;El-Adawy, 2002). In 2012, Amoo found that the increase in protein content of probioticated food products was due to the capacity of the microorganism's to produce amino acids during fermentation. The most widely used probiotic strains, *Lactobacillus casei* and *Lactobacillus plantarum*, have been clinically examined and determined to increase human natural resistance, support a healthy digestive tract, and hinder the adhesion of some pathogenic bacteria (WHO/FAO, 2011).

The ability of these probiotic strains to increase the amount of nutrients in food, such as protein, is due to the microbial synthesis of proteins from metabolic intermediates during their growth in the process of fermentation (Song *et al.,* 2012; Kumar *et al.,* 2015 Pandhare *et al.,* 2011; Nonogaki, 2010).

According to nutrient requirements for Indians, 2020 children till the age of 3 years should get protein of about 9.2 g/day from the diet. Hence the developed complementary food mixes fulfills the requirement of RDA for children.

Fat

The fat content of probiotic complementary food mixes CFM I and CFM II were 5.84±0.02 g and 6.60±0.05 g respectively as shown in Table 4.14. The fat content of probiotic complementary food mixes were significantly lower (P≤0.01) than the untreated ones T_4 (6.90 ±0.05 g) and T6 (7.00± 0.12g).

The results of the present study was similar to the work of Ikujenlola and Fashakin (2005) in complementary diets prepared from rice, maize, cowpea and sesame who reported a fat content of 5.50±0.23 and 7.10±0.06 g/100g respectively. However, studies reported by Elharadallou and Farh (2014) in complementary mixes made from local foods showed lower fat content of 5.0 and 5.8 %. This may be due to the addition of 10% sugar in the formulation and use of

pigeon pea which have lower fat content (1.7 g/100g) when compared to bengal gram (5.6 g/100g). Ahmed *et al*., (2008) in his study of complementary mixes made from wheat flour and soya flour also showed lower fat content of 3.52 to 4.88% which may be because they did not use any nuts or oilseeds in the formulation.

Ojokoh and Orekoya (2016) reported significant decrease in fat content in their study on the impact of lactic acid fermentation on the principal components of value-added products.They discovered that the fermented sample's fat content (6.82 ±0.37g) was lower than the unfermented sample's (7.04± 0.74g) per 100g of sample. The observed decrease in fat content in the fermented sample may also be the result of lipolitic microorganisms present in the sample breaking down fatty acids and glycerol, which releases a significant amount of energy during fermentation. Lipolitic microorganisms can break down both vegetable and animal fats (Ojokoh and Babatunde, 2014).

Crude fibre

The crude fibre values of CFM I and CFM II were 5.62 ± 0.02 g and 6.12 ± 0.07 g respectively (Table 4.14). The results showed significant decrease (P≤0.01) in crude fibre content of CFM I and CFM II as compared to T_4(10.00± 0.02 g) and T_6 (11.59 ±0.10 g)

The higher fibre content in both the formulations in the present study can be due to the *in situ* composition of the raw ingredients containing high fibre used for the study. Numerous studies showed that functional foods such as legumes, millets, and oilseeds when included in the diets significantly increasedaily dietary fibre consumption (Iqbal *et al.,* 2006; Lohia and Udipi, 2015). The fermenting organisms (lactic acid bacteria) capacity to metabolise the readily accessible fibre by enzymatically breaking them down during fermentation and using them as a carbon source may be the cause of the drop in crude fibre content (Oboh and Akindahunsi, 2014).

In 2008, Roberts and Knorr reported that the growth and multiplication that takes place during fermentation of *Lactobacillus casei* and *Lactobacillus plantarum* adheres strongly to the dietary fibres present in the food, which creates

a possibility of decreased fibre content probiotic food products developed using cereals and legumes. Similar findings on fermented bombara groundnut seed flour were made by Ogodo *et al.,* (2018). They discovered that the fibre content considerably reduced (P≤0.001) throughout the fermentation process using lactobacillus bacteria.

Total mineral

The total mineral content of CFM I and CFM II were 1.23 ± 0.01 g and 2.00 ± 0.05 g respectively. The results showed significant increase (P≤0.01) in total mineral content of CFM I and CFM II compared to T_4 (2.50 ±0.10 g) and T_6 (4.00 ±0.05 g).The total mineral content of T_4 and T_6 was not significantly different.

The increase in total minerals after probiotification, may be due to the process of fermentation, as during fermentation process, a partial consumption of minerals by fermenting microorganisms occurs during metabolism of lactic acid bacteria. This observation is related to the research of Ojokoh and Babatunde, 2014, who stated that the increase in mineral content of millet-soyabean blends is caused by incomplete utilization of minerals by fermenting organisms during their metabolism.

Based on the results of the proximate composition of the developed Probiotic complementary food mixes CFM I and CFM IIit was confirmed that moisture, protein and fat values were within the values recommended in RDA, 2020 and BIS, 2006. Therefore, the null hypothesis'H_0 - The Probiotification of complementary food mixes will not significantly improve the functional and nutritional quality of mixes' was rejected.

4.4.5. Mineral composition of the Probiotic Complementary Food Mixes

Data pertaining to the mineral composition of probiotic complementary food mixes (CFM I and CFM II) are presented in Table 4.15. Nine different minerals such as sodium, potassium, calcium, magnesium, manganese, iron, zinc, copper, phosphorus were analyzed.

Table 4.15. Mineral composition of the probiotic complementary food mixes

Minerals (in ppm)	CFM I	CFM II
Sodium	2.32±0.26	5.72±0.24
Potassium	235.18±1.02	432.38 ±0.3
Calcium	87.53±0.40	259.58±0.38
Magnesium	6.02±0.02	6.85±0.30
Manganese	9.85±0.30	15.48±0.42
Iron	10.26±0.21	13.65±0.52
Zinc	4.31±0.35	4.84±0.14
Copper	11.49±0.45	14.15±0.21
Phosphorus	40.91±0.38	55.11±0.99

Values are expressed in mean ± SD (Standard Deviation),

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

Sodium

Rice powder and other cereals (are theoretically advantageous, because they contain amino acids or carbohydrate (in the form of glucose chains), helps in promoting sodium transport. Additionally, because the glucose is in the form of starch, more can be given without increasing the osmolarity hence it could increase the absorption of sodium and water and decrease the diarrhoeal volume and duration of diarrhoea.

Sodium content for CFM I and CFM II was 2.32 ± 0.26 ppm and 5.72 ± 0.24 ppm respectively.

Potassium

Potassium content for CFM I and CFM II was 235.18±1.02 ppm and 432.38±0.33 ppm respectively. Harrison (1990) did the important determination and found out that not only sodium supplementation as the important treatment of diarrhoeal dehydration but also recognized the possible importance of potassium loss in diarrhoeal infants.

Calcium

Calcium content for CFM I and CFM II was 87.53±0.40 and 259.58±0.38 ppm respectively. A commonly shared phenomenon about homemade complementary foods that are based on starchy roots and tubers or rice and available in many low-income countries is their frequent shortfall in amounts of selected essential micronutrients such as calcium, iron, and zinc. In contrast, the recipes prepared from maize and legumes or other cereal mixtures and legumes had higher iron and zinc contents (Abeshu*et al.,* 2016).Riat and Sadana (2009) also found increase in calcium content of lactic acid fermented convenient mixes, they revealed that increased calcium content in fermented samples is due to the breakdown of phytate complex resulting in mineral bioavailability during fermentation. The study present was in conformity with the above findings.

Magnesium

Magnesium content for CFM I and CFM II was 6.02±0.02 ppm and 6.85±0.30 ppm respectively. Research by Ziegler *et al.,* (1976) showed that a newborn in the initial stage of life intakes, on average, 30 mg magnesium per day from the mother's milk and at 6 months of age, this quantity reaches 40 mg/day. Magnesium metabolism is closely associated with calcium metabolism. In breast milk the ratio of calcium to magnesium is 9:1, while in the commercial formulas, it was 4:1. This increased magnesium content may result from a smaller absorption of this element from formulas compared to mother's milk. In this situation, it appears that Mg deficiency in children fed with formulas is rather unlikely (Molska*et al*., 2014). Hence Magnesium content was estimated to confirm whether it fulfills the requirement of developed complementary mixes.

Manganese

Manganese content for CFM I and CFM II was 9.85±0.30 ppm and 15.48±0.42 ppm respectively.

Iron

Iron is key nutrient in human nutrition for growth and development. It is a component of muscle, blood and essential to carry oxygen around the human body. As iron participates in a large number of cellular processes, oxygen transport, adenosine triphosphate (ATP) generation, cell growth and proliferation.

Thus, the stored iron content in food mixes tends to decrease (Munasinghe*et al.,* 2013).

Iron content for CFM I and CFM II was 10.26±0.21 and 13.65±0.52 ppm respectively. Beyond the age of 6 months, more than 90% of the iron requirements of a breast-fed infant must be met by complementary food rich in bio-available iron. It is difficult to meet full iron requirements in young children through diet without fortifying complementary feeds or iron supplements (Shindey and Patel, 2014). As a public health measure, food fortification can play a major role in decreasing the prevalence of iron deficiency (Indian Academy of Paediatrics,2019). Significant iron deficiency in infants may lead to impaired cell-mediated immunity.With optimal reserves of iron in the newborn, the absorption of this micronutrient supplied with breast milk after birth is sufficient to cover the daily requirements of children. At about 4–5 months of age, an increasing demand of tissues for iron increases to about 0.5 mg/day (Molska *et al.,* 2014).

Zinc

Zinc is not well conserved in young child body as there is no conventional tissue reserve. Its status depends on regular dietary zinc intake. Low intake of zinc rich foods (animal or sea products), high intake of inhibitors (phytates) and losses in diarrhoea contribute to widespread zinc deficiency. It is more in protein energy malnourished children (Indian Academy of Paediatrics, 2019). For infants up to 1 year of age, the recommended daily amount of Zn is 5.0 mg/day, and in further development, it increases, depending on the sex of the child. The concentration of this element in natural mother's milk is about 2 mg/kg and decreases over time with lactation. After 3 weeks, it is only 36-54% of the initial concentration, and after 3 months, not more than 20%.

Zinc content for CFM I and CFM II was 4.31±0.35 ppm and 4.84±0.14 ppm respectively. The increase in the zinc contents of probioticated complementary food mixes during fermentation could be due to a special function of Lactic acid bacteria to uptake and bind zinc from the fermented medium (Hassan *et al.,* 2020; Leonardi *et al.,* 2013; Rosi *et al.,*2011) though at lower pH values during prolonged fermentation, the affinity of the cell wall for metal ions decreased.

Nuraida (2015) evaluated the effect of probiotic *Lactobacillus plantarum* on zinc content of traditional Indonesian fermented food 'Tempoyak' and it has been observed that microencapsulated *Lactobacillus spp.* at a dose of 10^6 cfu of inoculation in fermented foods showed a potential ability to improve the zinc status of the infants. They also found that supplementation of such probioticated food resulted in a significantly increased humoral immune response, as well as improved zinc status. The present results were supported by findings mentioned above.WHO (2001) designates calcium, iron, and zinc as 'problem nutrients', and slight deficiencies of these minerals can lead to adverse health consequences and restricted child growth and development.

Copper

Full-term human infants are believed to possess adequate copper stores to last through complementary regardless of the copper content of the diet they are fed. This may not be generally true, however; a combination of low copper intake and low bioavailability from the diet may lead to copper deficiency (Lönnerdal, 1998).

Copper content for CFM I and CFM II was 11.49±0.45 ppm and 14.15±0.21 ppm respectively. BIS (2006) gave the minimum requirement of copper in complementary food mixes as 2.8 ppm to 15.0 ppm.

Phosphorus

Calcium and phosphorus are an essential part of child's diet, since most of the growth and development that takes place starts from a young age and requires proper proportion of all nutrients. It has been suggested that the calcium to phosphorus ratio (Ca:P) is important for bone growth and development during infancy. (Sax, 2001; Bass & Chan, 2006).Phosphorous content for CFM I and CFM II was 40.91±0.38 ppm and 55.11±0.99 ppm.

Based on the above results it was observed that, CFM II displayed highest levels of sodium (5.72± 0.24 ppm), potassium (432.38±0.33 ppm), calcium (259.58±0.38), magnesium (6.85±0.30 ppm), manganese (15.48±0.42 ppm), iron (13.65±0.52 ppm), zinc (4.84±0.14 ppm), copper (14.15±0.21 ppm), phosphors (55.11±0.99 ppm). CFM I displayed comparatively lower levels of sodium,

potassium, calcium, manganese, iron, zinc, copper, phosphorus, magnesium compared to CFM II.

Mineral content of the developed complementary food mixes in the present study satisfied the recommended nutrient intake for age group 6 to 24 months by WHO (WHO, 2013). The result of the study of mineral composition showed that the micronutrient density is adequate for the healthy growth and development of children (age 6-24 months).

4.4.6 Free radical scavenging activity (RSA) of the probiotic complementary food mixes

The scavenging ability of the probiotic complementary food mixes have been studied using a stable free radical DPPH (1,1-diphenyl-picryl hydrazyl radical) having a purple colour. DPPH is well known radical and a trap ('scavenger') for other radicals (Visioli *et al.,* 2000). When free radical scavengers are added, DPPH are reduced and its color is changed to yellow, based on the efficacy of antioxidants. The test for free radical scavenging activity has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto *et al.,* 2000).

The free radical scavenging activity of developed CFM I and CFM II are shown in Fig.4.7.

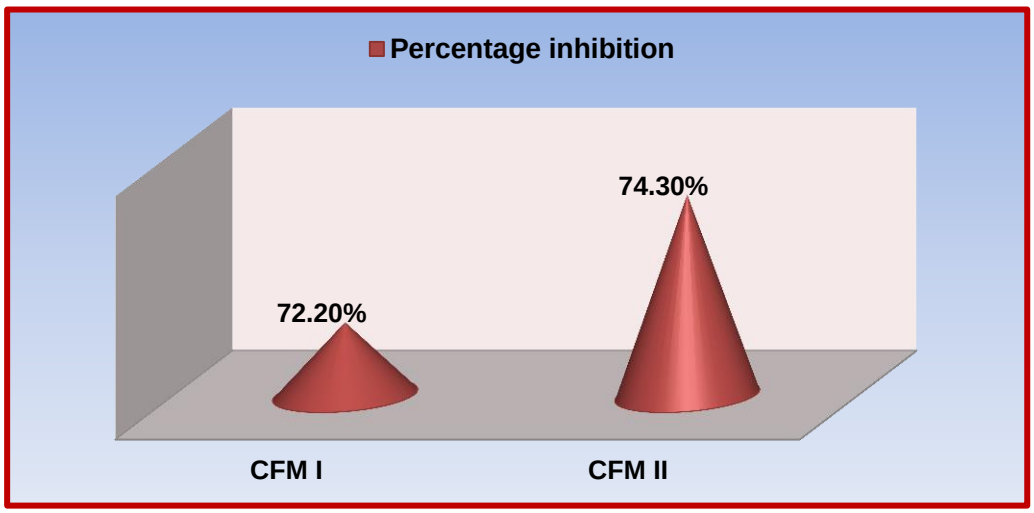


Fig.4.7. Free radical scavenging activity of probiotic complementary food mixes

The free radical scavenging activity of developed CFM I and CFM II were 72.2% and 74.3% respectively. Highest free radical scavenging activity was observed in CFM II

Caili *et al.,* (2014) reported an increase in free radical scavenging activity from 64.66% to 66.71% after probiotification of 'Kombucha' – a food mix – when inoculated with Lactobacillus bacteria. Persichetti *et al.,*(2014) and Roy *et al.,* (2013) stated that lactic acid probiotic bacteria possess significant free radical scavenging activity in non-dairy food products by both *in vivo* and *in vitro* studies and also found increased free radical scavenging activity in lactic acid fermented cereal-legume based food products. As for fermenting the developed complementary food mixes Lactobacillus strains were employed, the findings were in agreement with the present findings.

Several other studies also summarized that fermentation with *Lactobacillus plantarum*has a positive influence on phytochemicals of functional ingredients which leads to higher antioxidant activity (Katina *et al.,* 2007; Zhang *et al.,* 2008). They reported that during fermentation process, structural break down of cell walls may occur, leading to the increased extraction of bioactive compounds which are responsible for increased antioxidant capacity (Sreeyan *et al.,* 1996; Heinio *et al.,* 2003; Balasundram *et al.,* 2006). The action of enzymes such as β-glucosidase, α-amylase and lactase secreted during the process of fermentation of food products by lactic acid bacteria in the improvement of antioxidant capacity (Hidalgo-Cantabrana *et al.,* 2012; Zheng and Shetty (2000).

Xing *et al.,(2015) and* Afifi *et al.,* (2012) found that *Lactobacillus casei* showed high antioxidative effect when compared to other strains of *Lactobacillus* and also revealed that high antioxidant capacity in biscuits incorporated with *Lactobacillus casei* when estimated by DPPH method. Dordevic *et al.,*(2010)and *Zhou et al.,*(2016) also showed that fermentation of several cereals (buckwheat, barley, wheat, rye) by *Lactobacillus casei* for 24 hours increased the total antioxidant activities measured by DPPH method.

Many researchers stated that *Lactobacillus plantarum*and *Lactobacillus Casei*contains Exopolysaccharides (EPS), a 'food grade biopolymers'obtained

from natural sources that are formed during the metabolic process of these microorganisms which contribute potential health promoting properties. These compounds exhibit potent antioxidant efficacy by interacting with similar components present in fermentation medium (Zhou *et al.,* 2016; Seo *et al.,* 2015; Gangoiti *et al.,* 2017). Lactobacillus strains derived EPS can protect the microbial cells against desiccation, phagocytosis, phase attack, antibiotics or toxic compounds (Liu *et al.,* 2011; Polak *et al.,* 2013).

From the above study it can be concluded that both the formulations CFM I and CFM II had free radical scavenging activity but highest free radical scavenging activity among the twowas observed in CFM II.

4.4.7. Qualitative and quantitative phytochemical profile ofprobiotic complementary food mixes

The qualitative and quantitative phytochemicals profile ofprobiotic complementary food mixes are given in Table 4.16.

Table 4.16. Qualitative and quantitative phytochemical profile ofprobiotic complementary food mixes

Nutraceuticals component	CFM I	CFM II	CFM I	CFM II
Flavonoids (quercetin/g)	+	+	107.96±0.83	130.00±0.95
Alkaloids (atropine/g)	+	+	23.60±0.36	41.06±0.51
Cyanogenic Glycosides (mg/g)	+	+	16.18±0.18	26.53±0.45
Tannins (tannic acid/g)	+	+	4.74±0.01	6.29±0.06
Oxalates (mg/g)	+	+	0.47±0.01	0.55±0.01
Trypsin inhibitor (TIU mg)	+	+	2.59±0.02	4.20±0.25
Saponins (diosegenin /g)	+	+	9.01±0.01	13.41±0.07
Phytate (mg/100 g)	+	+	0.24±0.02	1.68±0.03

Values are expressed in mean ± SD (Standard Deviation).

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

From the above data it was observed that phytochemical profile of the developed probiotic complementary food mixes exhibited secondary metabolites namely flavonoids, alkaloids, terpenoids, saponin, and cyanogenic glycosides, tannins, oxalates, trypsin inhibitor, saponins and phytate.

Phytochemicals compounds exist as a natural component of cereals, legumes and other plant foods (Roos *et al.,* 2013).

From the above results it was observed that the phytochemical components were higher in CFM II as compared to CFM I. Flavonoids content of CFM II was 130±0.95 quercetin/100g. Alkaloids content was 41.06± 0.51 Atropine/100g. Cyanogenic glycosides content was CFM II 26.53 ± 0.45 mg/100g. Tannins content was 6.29±0.06 tannic acid/100g. Oxalates content was 0.55±0.01 mg/100g. Trypsin inhibitor content was 4.20±0.25 TIU/100g. Saponins content was 13.41±0.07 diosegenin/100g and phytate content was 1.68±0.03 mg/100g respectively.

As the developed complementary food mixes in the present study were for infants aged 6-12 months.Phytonutrients analysis plays a major role in the formulation of the mixes, as these could impart negative changes in the growth of children. Studies have confirmed that gain in body weight and food utilization efficiency is lower when the food contains considerable amounts of phytonutrients agents (Gilani *et al.*, 2005).

In the present study, one fourth of the formulation of the developed complementary food mixes was legumes for the increasing the overall protein, fat and nutritional value of foods, but on the contrary it contains many anti-nutritional factors like trypsin inhibitors, saponin, lectin etc. as quoted by Liener (1994). Phytates, oxalates, and tannins are found in most of the plant based foods. Most of the phytonutrients factors are reduced or inactivated by traditional cooking and food processing. This improves flavor, palatability and increases the bioavailability of nutrients (Gebrelibanos *et al.*, 2013). Hence, in agreement of the above findings, the traditional cooking methods were employed in the present study to enhance the organoleptic properties.

Awoyinka (2016) and Adeyemo and Onilude (2013) found significant decrease in saponin content (from 0.87% to 0.03%) of wild edible bean (Mucuna) of Nigeria when fermented with *Lactobacillus plantarum*. It was also reported that *L. plantarum*, played a significant role in the reduction of anti-nutritional compounds and detoxification of harmful substances in food during fermentation. *Lactobacillus plantarum* and *Lactobacillus casei* has been used for centuries in preservation of food for increased shelf life and flavours (Daeshel, 2004; Harris, 2010). Many studies revealed that lactic acid fermentation are the safest way to preserve food. These are one of the most versatile probiotic and have also been implicated in the lowering of anti-nutrients components in food during fermentation. Hence in the present study both*Lactobacillus plantarum* and *Lactobacillus casei*was utilized for fermentation of complementary food mixes in accordance with the present study.

From the above results it was observed that the flavonoids content, Alkaloids content,Cyanogenic glycosides, Tannins content,Oxalates, Trypsin inhibitor,Saponins content and Phytate content was found to be in higher amount in CFM II as compared to CFM I.

4.4.8. *In-vitro* protein and starch digestibility of the probiotic complementary food mixes

4.4.8.1. *In-vitro* protein digestibility of the probiotic complementary food mixes

In-vitro protein digestibility of the probiotic complementary food mixes are presented in Table 4.17.

Table 4.17. *In-vitro* protein digestibility of the probiotic complementary food mixes

Treatments	*In-vitro*protein digestibility (%)
CFM I	73.46±0.50
CFM II	70.26±0.37

Values are expressed in mean ± SD (Standard Deviation),

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

According to Codex standards (1991), protein digestibility content in a complementary food mixes should not be less than 70 percent per 100g mix.The protein digestibility of the probiotic complementary food mixes of CFM I was 73.46±0.50% and CFM II was 70.26±0.37% (Table 4.17).

The results of protein digestibility of the present study were in accordance with the codex standard (1991). The findings of the present study are similar with the work done by Usha *et al*., (2010), who reported protein digestibility of 71.8% in a complementary mix prepared from rice, sorghum, malted green gram and pumpkin flour. Kshirasagar *et al*., (1994) also reported 70.98% protein digestibility of complementary mix prepared from malted ragi which is in accordance to the present study. Contrary to the findings, studies reported by Ali (2016) who formulated complementary mix from maize and pulses by using extrusion technology reported protein digestibility (89%) higher than the present study which could possibly be due to extrusion cooking which improved the protein digestibility due to protein denaturation and inactivation of the anti-nutritional factors during the extrusion process.

The values for protein digestibility of protein in millet based diet were 63 to 65 per cent (Hopkins *et al.,* 1981). Several studies have proven that protein digestibility, decreased up to 40 per cent, in animals fed diet containing raw soybean compared to those containing roasted soybean (Gilani et al., 2005, Li *et al.,* 2000). Although the anti-nutrient factors present are generally inactivated by heat during cooking, complexes formed between these substances and protein may not be completely dissociated. It may interfere with the protein digestion (Rockland *et al.,* 1981).

Table 4.18. Correlation between viscosity and protein digestibility

Pearson correlation coefficient			
		Viscosity	**Protein digestibility**
Viscosity	Pearson Correlation	1	.698**
	Sig. (2-tailed)		.006
Protein digestibility	Pearson Correlation	.698**	1
	Sig. (2-tailed)	.006	
** Correlation is significant at the 0.01 level (2-tailed)			

Considering the acceptability of developed complementary food mixes by the above results, the assessment of the relationship between viscosity and protein digestibility was done by Pearson product-moment correlation coefficient and it was revealed that there was a significant positive correlation between the viscosity and protein digestibility(r=0.698, p=0.006) which is supported by Gerald *et al.,* (2018) who reported that fermentation time had a positive linear effect on the protein digestibility and viscosity. The correlation between viscosity and protein digestibility are shown in Fig. 4.8.

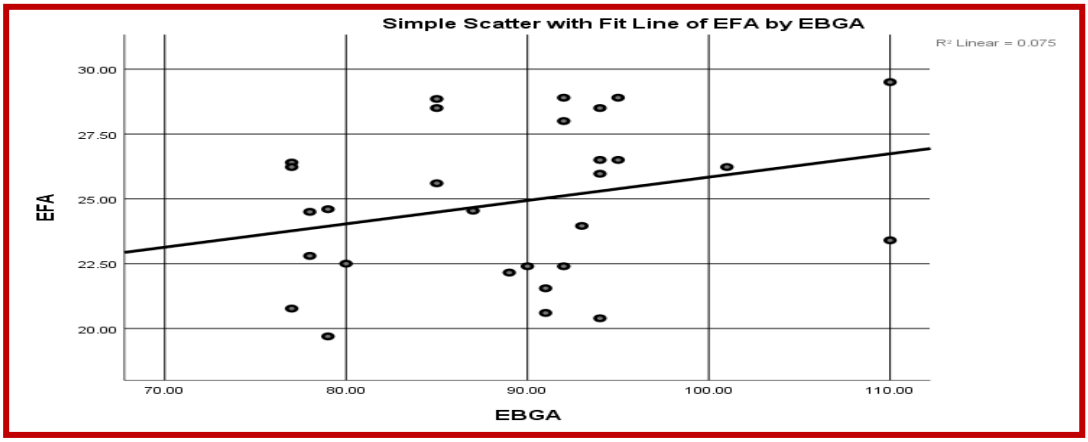


Fig. 4.8. Correlation between viscosity and protein digestibility

4.4.8.2. *In-vitro* starch digestibility of the probiotic complementary food mixes

Data pertaining to enzymatically assessed total starch, digestible starch (DS), rapidly digestible starch (RDS), resistant starch content and starch digestion index is presented in Table 4.19.

Table 4.19. *In-vitro* starch digestibility of the probiotic complementary food mixes

Treatments	Total starch (%)	Resistance starch (%)	Digestible starch (%)	Rapidly digestible starch (%)	Starch digestion index (%)
CFM I	78.72±0.50	2.06±0.03	70.23±0.23	20.88±0.68	20.72±0.63
CFM II	61.03±0.50	2.17±0.02	65.05±0.05	19.11±0.10	20.84±0.29

Values are expressed in Mean±SD (Standard Deviation),

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

Starch is known to possess low digestibility. Starch digestion index is an important parameter for comparing starch digestibility. Rate of digestion depends on various factors like amylose content, starch protein interaction, level of resistant starch etc. Rapidly digestible starch (RDS) is the completely digested starch (within 20 minutes of incubation period). Resistant starch is the sum of starch and products of starch degradation. Resistant starch is not digested and not absorbed in small intestine. It gets fermented in large intestine.

The total starch of CFM I was 78.72±0.50% and 61.03±0.50%, resistance starch of CFM I was 2.06±0.03% and CFM II was 2.17±0.02%, digestible starch of CFM I was 70.23±0.23%, and CFM II was 65.05±0.05%. Rapidly digestible starch of CFM I was 20.88±0.68% and CFM II was 19.11±0.10% and starch digestion index for CFM I was 20.72±0.63% and CFM II was 20.84±0.29%.

Fig.4.9. shows the *In-vitro* starch digestibility of the probiotic complementary food mixes in CFM and CFM II.

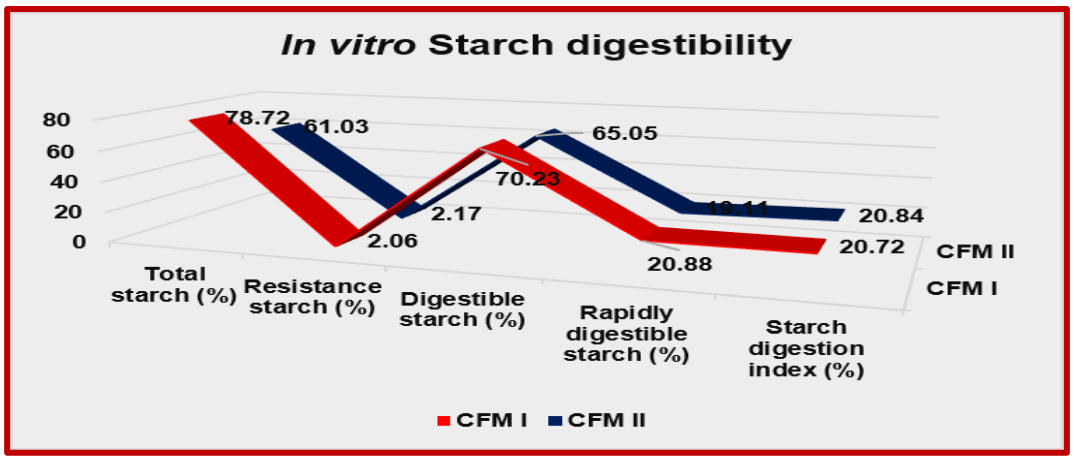


Fig.4.9.*In-vitro* starch digestibility of the probiotic complementary food mixes

The findings of the present study were similar to the work done by Kshirasagar *et al.,* (1994) who formulated a complementary mix from ragi, green gram and groundnut powder and reported a total starch digestibility of 70% per 100 g of sample. Studies reported by Oumarou *et al.,* 2005 on complementary foods made from processed and fermented sorghum, groundnut, spinach and mango showed a significant increase in starch digestibility from 76.87% - 92.25%

to 83.49% - 98.14% as a result of pre-treatment of grains and fermentation of flours. Highest increase was observed in germinated and fermented samples and lowest was observed in non-processed flours. The amount and type of fatty acid present in millets also affect starch digestibility (George *et al*., 2017). The findings were in conformity with the present study.

From the above findings it can be concluded that the total starch (%), resistance starch (%), digestible starch (%), rapidly digestible starch (%) and starch digestion index (%) was found to be highest in CFM I when compared with CFM II.

PHASE V

4.5. Acceptability trials of the probiotic complementary food mixes

Acceptability trials were conducted by trained and semi trained panel consisting of 30 members from the Department of Food Science and Nutrition in the sensory evaluation laboratory of Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and shown in Table 4.20.

Table 4.20. Acceptability trials of the probiotic complementary food mixes

Sensory attributes	T_4	T_6	CFM I	CFM II	CD $_{0.05}$
Colour	6.33±0.72^{a}	7.10±0.20^{b}	7.36±0.22^{c}	8.43±0.43^{d}	0.114**
Flavour	6.58±0.58^{a}	7.38±0.38^{c}	7.56±0.40^{b}	8.00±0.34^{d}	0.113**
Consistency	6.53±0.43^{a}	8.08±0.18^{c}	8.53±0.83^{b}	8.35±0.39^{d}	0.132**
Appearance	6.90±0.35^{a}	7.08±0.37^{b}	7.55±0.35^{a}	8.38±0.44^{b}	0.099**
Taste	6.83±0.35^{a}	7.00±0.58^{d}	7.78±0.25^{b}	8.06±0.34^{c}	0.103**
Overall Acceptability	6.66±0.79^{a}	6.83±0.46^{a}	7.80±0.33^{b}	7.98±0.44^{b}	0.137**

A- T_4, B-, T_6, A1- CFM I, B1- CFM II

Values are Mean±SD of 30 replications **Means with different superscript within the same row are significantly different at p≤0.05

T_4 = *Rice* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%, T_6 = *Finger millet* 50% + Mung bean 25% + MWF 5%+Sesame Seeds 10% + Pumpkin Seeds 10%, CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

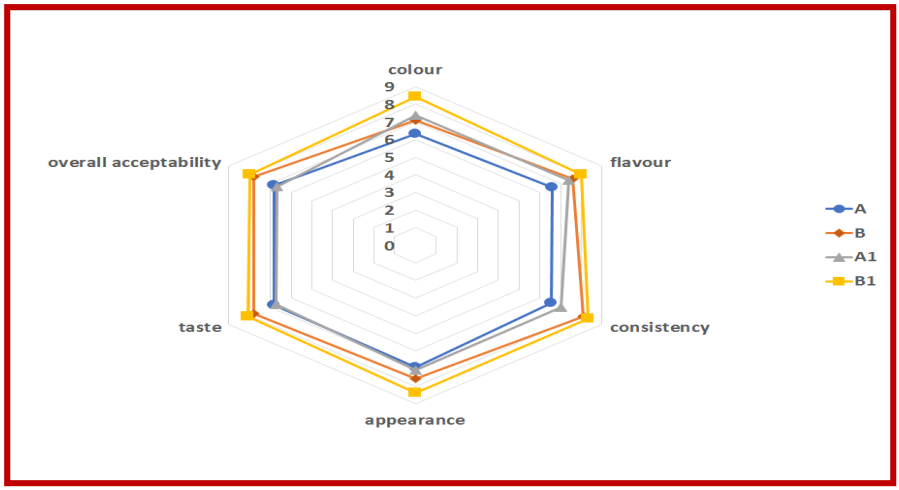


Fig 4.10. Mean organoleptic scores between different formulated mixes

The sensory parameters in each formulation scored significantly different scores at ($p \leq 0.05$). CFM II scored highest (8.43±0.43) and T_4 scored lowest (6.33±0.72) in colour; CFM II scored highest (8.00±0.34) and T_4 scored lowest (6.58±0.58) in flavour; CFM I scored highest (8.53±0.83) and T_4 scored lowest (6.53±0.43) in consistency; CFM II scored highest (8.38±0.44) and T_4 scored lowest (6.90±0.35) in appearance; CFM II scored highest (8.06±0.34) and T_4 scored lowest (6.83±0.35) in taste; CFM II scored highest (7.98±0.44) and T_4 scored lowest(6.66±0.79) in overall acceptability.

As the fermented food mixes were very well accepted by the panel members, The findings were in agreement with the findings of Kazeem and Wakil (2021) and Zema *et al.,* (2015) stating that, lactic acid and other metabolic products contribute to organoleptic and textural profile of the food item. Samples fermented with *L.plantarum* have a pleasant odour, taste and aroma longer shelf life.

The raw ingredients enhancing the organoleptic qualities of the developed mixes in the present study is supported by study conducted by Semwal *et al.,*(2015) who developed a complementary mix from underutilized crops of Uttarakhand (finger millet, barnyard millet, black soybean, amaranth grain)by using probiotic strain *Lactobacillus plantarum*. The probiotic fermentation resulted in favorable changes in sensory evaluation of complementary mix and had significant difference from the untreated samples. Plate 26 and Plate 27 show the formulated mixes and the prepared porridges.

Patent filed and published, Indian Patent Office, Chennai (202241007163/03/04/2021)

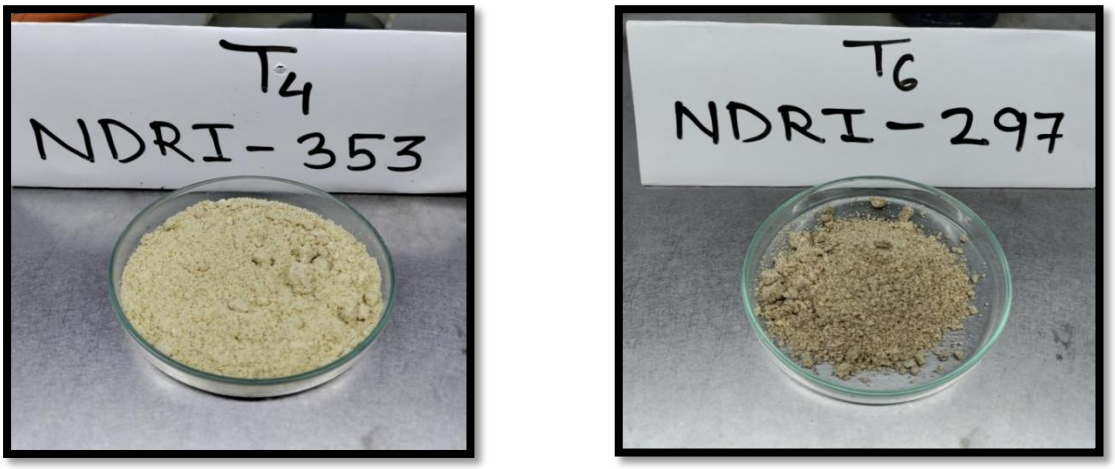


Plate 26. Freeze dried samples after fermentation (CFM I and CFM II)

Plate 27. Preparation of probiotic complementary food mixes

Simwaka (2017) reported the effect of natural fermentation on complementary foods formulated from pumpkin seeds, amaranth, finger millet and sorghum grains and found increased organoleptic properties of the developed complementary foods in comparison to the untreated ones.The study conducted by Isingoma *et al.,* (2015) aimed at improving the nutritional value and safety of finger millet porridges using lactic acid fermentation. The findings are inconformity with the present study. Sadana and Chabra (2004) and Tiencheu *et al.,*(2016) stated that factors like the chemical composition of the food mixes, the drying temperature and duration, and the proportions or ratio of ingredients can affect the sensory qualities of the composite diets.

All the four complementary food mixes were very well accepted by the panelists. Out of the four formulations prepared, the best scored formulation was CFM II in terms of flavor, appearance, taste and overall acceptability. CFM I scored highest in consistency. Therefore, it could be concluded that the addition of probiotic cultures on the mixes had a positive effect on the overall acceptability of the probiotificated mixes.

4.6. Assessment of functional efficacy of probiotic complementary food mixes through I*n-vivo* animal studies

4.6.1. Efficacy of probiotic complementary food mixes on the castor oil induced diarrhoeal Wistar strain albino rats

The efficacy of probiotic complementary food mixes on the castor oil induced diarrhoeal Wistar albino rats are presented in Table 4.21.

Table 4.21. Efficacy of probiotic complementary food mixeson the castor oil induced diarrhoeal Wistar strain albino rats

Treatment	Supplementation period (0-7 days)					
	Days of faecal collection (n=6)					
Experimental Groups (n=6)	**The onset of diarrhoea (min)**	**Total number of feces**	**Average weight of total feces (g)**	**Average fecal water content (g)**	**Total fecal output (%)**	**Reduction (%)**
Control	-	6.60±0.26	0.211±0.001	0.021±0.001	-	-
Negative control	21.38±0.57	15.99±0.08**	0.59±0.01**	0.060±0.052**	76.52^{e}	13.02^{b}
Positive control	119.70±0.72**	4.48±0.03**	0.22±0.02**	0.019±0.001**	34.03^{b}	70.61^{e}
Experimental group A	87.34±0.56^	6.84±0.01**	0.33±0.01**	0.199±0.14**	56.30^{d}	64.86^{c}
Experimental group B	100.47±0.89+	6.17±0.01**	0.238±0.001**	0.021±0.001**	50.19^{c}	68.91^{d}

NC- Negative control group, diarrhoeal induced fed with rat ration, PC-Positive control group, diarrhoeal induced fed with rat ration along with commercial drug, Experimental group A- fed with CFM I, Experimental group B- fed with CFM II.CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

Values are expressed in Mean±SD (N=6) **p<0.01 compared with NC group, ^ p<0.01 compared with Group B and + p<0.01 compared with group A. Means within columns separated by Duncan's multiple range test p = 0.01

In the present study castor oil induced diarrhoeal model was used to study the whether probiotic complementary food mixes significantly reduced the onset of diarrhoea along with it reduced the frequency and weight of total stool compared with the negative control. All the probiotic fed groups showed statistically

significant difference ($p<0.01$) effects on the onset of diarrhoea, the total number of feces, the average weight of total feces, and average fecal water content compared to the negative control group.

The results revealed that the percentage of diarrhoeal inhibitions were 13.02% for negative control group, 70.61% for positive control group and 64.86% for experimental group A and 68.91% for experimental group B respectively.

Experimental group A and experimental group B showed significant ($p<0.01$) differences with positive control regarding onset of diarrhoea. Moreover, a significance difference ($p<0.01$) was observed in onset of diarrhoea when compared with Group A and B. The total number of feces was significantly ($p<0.01$) reduced in all of thegroups compared with the negative control group. Total stools weight in positive control group, Group A and Group B group showed significant ($p<0.01$) reduction compared negative control group. Probiotics exhibiting anti-diarrhoeal activity may have a potential to retard the onset of diarrhoea significantly as seen in Group A and Group B.

The present findings werein concordant with the studies conducted by Gidudu *et al.,* (2011) and Jungersen *et al.,*(2014) in which *Lactobacillus sporogenes* significantly reduce the episodes ($p<0.002$) and shorten the duration ($p<0.001$) of acute rotavirus diarrhoea in infants than placebo.Oral administration of probiotic bacteria to preterm neonates was reported to decrease the all-cause mortality and in particular the incidence of diarrhoea in a number of studies (Van Best *et al.,* 2020; Zivkovic *et al.,* 2011; *Sela et al., 2012;* Yatsunenko *et al*., 2012).

Foster *et al*., (1980) reported that, capsules of *Lactobacillus* preparation in infected ileal loop significantly reduced the enterotoxin response against *Escherichia coli* enterotoxin induced diarrhoea in the rabbit. Also *Lactobacillus* GG reduced the duration of non-bloody diarrhoea compared to the control (31% vs 75% at 48 h) admitted for severe diarrhoea and malnutrition. Hence, Lactobacillus spp. can be considered effective in reducing the infection caused by *Escherichia coli* leading to diarrhoea. Therefore, in the present study strains of lactobacilli was used for fermenting the mixes and study its effectiveness in anti-diarrhoeal Wistar strain albino rats.

The results from the above study revealed that the percentage of diarrhoeal inhibitions were lowest for negative control group, and significantly higher for positive control group, Experimental group A and experimental group B respectively.Therefore, the null hypothesis 'H_0 -Supplementation of developed probiotic complementary food mixes to Wistar albino rats will not significantly inhibit the diarrhoeal episodes of experimental rats' was rejected.

4.6.2. Impact of feeding probiotic complementary food mixes (CFM I and CFM II) on the gut microflora of Wistar strain albino rats

The impact of feeding probiotic complementary food mixes (CFM I and CFM II) on the gut microflora of Wistar strain albino ratsare presented in Table 4.22.

Table 4.22. Impact of feeding probiotic complementary food mixes on the gut microflora of Wistar strain albino rats

Days of fecal collection	Population count (10^8 cfu/g)					
	Experimental Groups (n=6)					
	Control (Rat ration)		Experimental Group A		Experimental Group B	
	Total microbial load	Probiotic cells	Total microbial load	Probiotic cells (CFM I)	Total microbial load	Probiotic cells CFM II
0 (Feeding day)	10.6	-	10.2	-	11.6	-
1	10.5	-	9.6	5.8	9.5	6.7
2	10.5	-	8.8	7.2	8.2	8.0
3	10.6	-	6.9	7.4	6.4	8.7
4	10.6	-	7.0	7.8	6.3	9.2
5	10.6	-	6.5	6.6	5.2	8.4
6	10.6	-	5.4	5.8	4.5	7.8
7	10.5	-	4.2	5.1	4.3	6.5
				f-value	**SEd**	**CD at 5%**
For factor time				61.2**	5.2	4.2
For factor treatment (microbial load)				54.2**	6.3	3.8
For factor treatment (probiotic cells)				43.7**	6.8	3.5
For factor time × treatment (microbial load)				31.8**	8.7	6.1
For factor time × treatment (probiotic cells)				24.5**	9.5	7.5

Values are expressed in Mean±SD (N=6), ****** Signifcant at P<0.01

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

The present study was an aim to observe the rate of multiplication of probiotic bacteria over microbial growth during the seven days of the study period within the gut microflora of Wistar strain albino rats.

From the above presented data it can be revealed that the initial total microbial load of experimental group A was 10.2×10^8 cfu/g. A significant decrease ($p\leq0.01$) in total microbial load was observed from a total count of 9.6×10^8cfu/g on the 1st day to 4.2×10^8cfu/g on 7th day. The initial probiotic cell count in experimental group A after feeding with CFM I was found to be 5.8×10^8cfu/g on 1st day after feeding. There was a significant ($p\leq0.01$) gradual increase in the probiotic cell count of 7.8×10^8cfu/g till 4th day of supplementation period. Thereafter, a significant ($p\leq0.01$) decrease (5.1×10^8cfu/g) in probiotic cell count was observed till the end (7th day) of the feeding trial.

Similarly, the initial total microbial load of experimental group B was 11.6×10^8 cfu/g. A significant decrease ($p\leq0.01$) in total microbial load was observed from a total count of 9.5×10^8cfu/g on the 1st day to 4.3×10^8cfu/g on 7thday. The initial probiotic cell count in experimental group B after feeding with CFM II was found to be 6.7×10^8cfu/g on 1st day after feeding. There was a significant ($p\leq0.01$) gradual increase in the probiotic cell count of 9.1×10^8cfu/g till 4th day of supplementation period. Thereafter, a significant ($p\leq0.01$) decrease (6.5×10^8cfu/g) in probiotic cell count was observed till the end (7th day) of the feeding trial.

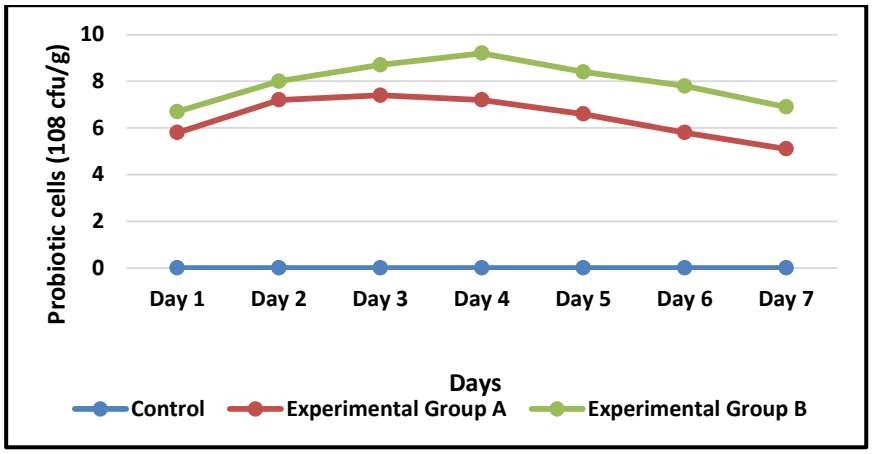
Vandenplas *et al.,* (2015) stated that probiotic products should have the viabile microorganisms until the time of consumption, their survival in food after exposure to gastrointestinal tract (GIT) conditions is crucial. They must arrive viable and active to different parts of intestine to adhere and colonize. The health benefits of a probioticated food are obtained only when a probiotic strain reaches the target site in a metabolically active state and in sufficient numbers. The results were in accordance with the present study.

Sato *et al.,* (2017) also found that probiotic strains like *Lactobacillus casei, Lactobacillus reuteri, and Lactobacillus gasseri,* significantly reduce the

pathogenic bacteria present in gut microflora when estimated through fecal microbial count in rats for ten days supplementation study.

Hence, probiotic strains present in both the formulations CFM I and CFM II were effective in increasing the population of viable probiotic bacteria through oral delivery of mixes to Wistar strain albino rats. The *L. plantarum* and *L. casei*present in mixes were able to survivethroughout the gastro intestinal transit increasing the useful bacteria in the fecal microbial count.

The growth and multiplication of probiotic bacteria present CFM I and CFM II gradually reduce the microbial load containing pathogenic bacteria as shown in Fig.4.11.



Control – fed with rat ration
Experimental Group A- fed with CFM I, Experimental Group B- fed with CFM II

Fig. 4.11.Growth kinetics of probiotic cells from CFM I and CFM II in the gut microflora of Wistar strain albino rats

Spring *et al.,* (2000) and Duffy (2009) stated that competitive exclusion (CE) is a specific type of probiotic strategy that involves the addition of a non-pathogenic bacteria culture (probiotic) to the intestinal tract of host body through food in order to reduce colonization or decrease populations of pathogenic bacteria in the gastrointestinal tract. Probioticconstantly binds and removes pathogens from the intestinal tract by stimulating the host's immune system. The study was in accordance with the present findings were probiotic strains present in the formulations competed with the pathogens at fixation sites, thereby reducing the microbial load and increasing the probiotic viable cell count.

Both the experimental groups showed cell viability of 10^8 cfu/g till 7 days of supplementation period. The results were in conformity with the findings of De Vuyst (2000) and Vamanu (2014) stating that the minimum number of viable cells must be between 10^6 to 10^8 colony forming units (cfu)/g to yield positive effects on the general health status of the host. Therefore, it can be concluded that both the probiotic complementary food mixes (CFM I and CFM II) possesses viable cell count of 10^8cfu/g that was in permissible limit as mentioned above.

4.6.3. *In-vivo* protein quality assessment of developed probiotic complementary food mixes

The net protein ratio (NPR) of the experimental diets CFM I and CFM II and its impact on growth of the Wistar strain albino rats are presented in Table 4.23.

Table 4.23. Impact of feeding probiotic complementary food mixeson the net protein ratio of Wistar albino rats

Parameters	Experimental Group A	Experimental Group B	Experimental Group C	Experimental Group D
	Diets (n=6)			
	Reference diet (Casein diet)	Control diet (Maple syrup)	Test diet (CFM I)	Test diet (CFM II)
Total food intake (g/day)	13.00±0.28	15.00±0.25	15.00±0.33	14.00±0.39
Total protein intake (g/day)	15.01±0.10	0.60±0.43	10.04±0.09	11.89±0.25
Initial weight (g)	15.00±0.26	17.00±0.22	14.00±0.41	19.00±0.37
Final weight (g)	45.00±0.34	11.00±0.38	40.00±0.39	42.00±0.20
Total weight gain/loss (g)	30.00±0.23	-6.00±0.34	26.00±0.54	23.00±0.65
NPR	31.37±0.28	20.00±0.54	26.11±0.67	30.17±0.48
RNPR	70.00±0.28	48.34±0.28	60.69±0.35	63.25±0.56

Values are expressed in Mean±SD (N=6), ****** Signifcant at p<0.01
NPR= Net protein ratio; RNPR= Relative net protein ratio

The highest net protein intake (31.37±0.28 g protein) was observed in group A supplemented with reference diet consisting of casein. Group B (control)

supplemented with protein free diet showed lowest mean protein intake of 20.00±0.54 g protein. On the other hand, among all the experimental groups C and D, supplemented with test diets CFM I and CFM II, the highest net protein intake was observed in group D supplemented with test diet CFM II. The mean protein intake of group D was 63.25±0.56 g protein followed by group C fed with test diet CFM I. The mean protein intake of group C was 26.11±0.67 g.

The increase in body weight of experimental group fed CFM I and CFM II were due to its higher protein content present in the developed probiotic complementary food mixes. The present study was supported by the study done by Rizkall et al., (2004) who found that the increase in crude protein content after fermentation was attributed to the vigorous multiplication of live microorganisms within the food by secreting certain extracellular enzymes such as amylases, linamarases and cellulases during their breakdown in the fermentation medium.

Among different methods of evaluation of protein quality, NPR is most widely used method for assessing the efficacy of protein utilization by using weanling rats and its impact on growth and development (Millward *et al.,* 2008; Mensa *et al.,* 2001). NPR is termed as a biological method that assesses the efficiency of protein utilization on growing rats (Pellet and Young, 1980). It has been revealed that bioassays by the use of rats are the most appropriate procedure for the measurement of protein quality especiallynet protein ratio (NPR).Miao *et al.,* (2015) and Sievenpiper *et al.,* (2002) stated that probioticated and fermented food formulations had higher protein quality when assessed through net protein ratio. The results were in conformity with the present study.

Hence from the present it was reported that the significant increase in body weight of experimental group C and D, fed with CFM I and CFM II were due to its higher protein content present in the developed probiotic complementary food mixes when compared with the other two groups A and B.

4.6.4. Food conversion efficiency (FCE)in Wistar strain albino rats

The average weight gain, food intake and food conversion efficiency were calculated and presented in Table 4.24.

Table 4.24. Food intake, weight gain and food conversion efficiency in Wistar strain albino rats

Groups	Diet	Food intake	Weight gained(g)	FCE
I	**100% RR**	12.00^{a}±0.50	24.00^{a}±0.50	0.25^{b}±0.04
II	**CFM I**	14.50^{b}±0.50	30.35^{c}±0.56	$0.22^{a,b}$±0.02
III	**CFM II**	15.00^{b}±1.00	26.83^{b}±0.76	0.18^{a}±0.01

Values are Mean±SD of 6 replications

Means with different superscript within the same column are significantly different at p≤0.05

Food conversion efficiency (FCE) is a ratio measuring the efficiency with which food given to the animal is converted into mass gained by the animal body. It is a ratio of inputs to outputs. FCE is a function of the quality and ingredients of the food and the conditions in which the animals are kept. Since all the animals were kept in standard laboratory conditions, FCE will only depend upon the quality of the food.

Results revealed that highest weight gain and maximum food intake was observed in group III fed with CFM II, followed by group II and I. The difference in the food intake may be due to difference in taste and palatability of the diets which in turn accounts for difference in weight gained by the rats. The FCE was lowest in group III indicating better quality of the formulation when compared to the others. The feed conversion efficiency (FCE) is a measure of an animal's efficiency in converting food mass into increase in mass gained by the body. A low FCE is an indication of a high quality feed (Idoko *et al*., 2015).

The findings of the present study are similar to the work done by Farran *et al*., (2005) who reported that taste and texture of finished feeds influences intake in animals. He also stated that low feed intake and presence of antinutritional factors are responsible for lower growth performance in rats. Fig. 4.12. shows the body weight of Wistar albino rats fed with different experimental diets.

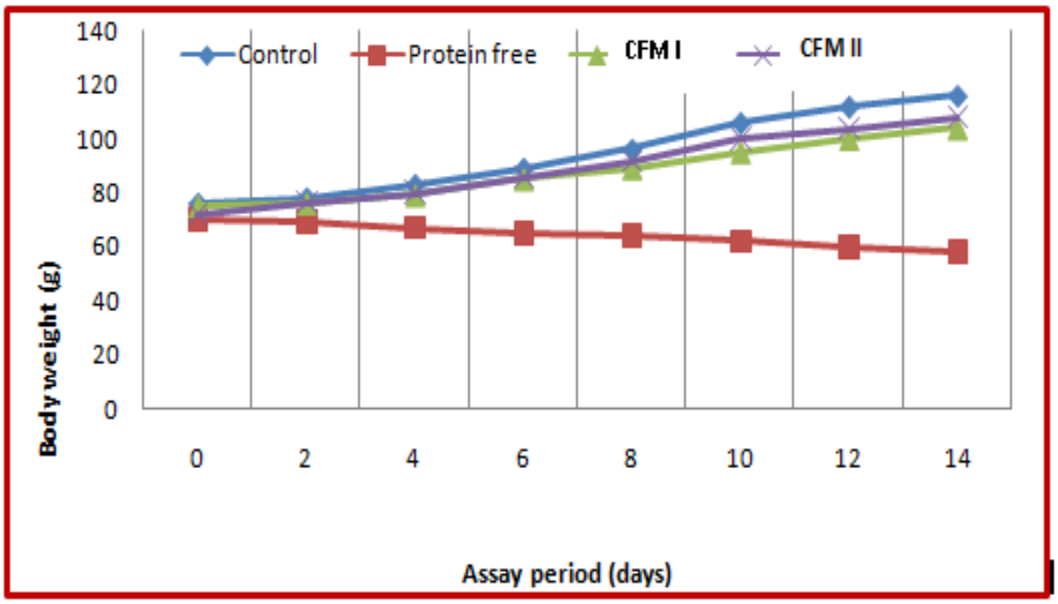


Fig. 4.12. Body weight of Wistar strain albino rats fed with different experimental diets for 14 days

The findings of the present study was supported by Ibironke (2014) that revealed that protein intake of a complementary food formulated from functional ingredients like rice, soyabeans and sesame seeds had the highest impact on the body weight of experimental weanling rats.In 2014, Monteiro reported that when a large meal of food containing high protein content is consumed, the net weight gain in experimental animal increases. Gernah *et al.,* 2012 stated that malting and fermentation tends to increase the quantity, quality and availability of nutrients due to hydrolysis of complex food reserves to simpler absorbable molecules.

Hence, it can be concluded from the above study that food containing high protein when given to Wistar albino rats showed positive results in terms of food intake and body weight.

4.6.5. Blood glucose levels and total blood protein levels of Wistar strain albino rats after feeding with probiotic complementary food mixes

Fasting blood glucose levels of the Wistar strain albino ratsare presented in Table 4.25.

Table 4.25. Blood glucose levels of Wistar albino rats

	Group I	Group II	Group III	CD0.05
DAY 0	$102.0^{a,b}$±2.61	113.11^{b}±0.34	100.83^{a}±0.76	NS
DAY 7	103.3^{b}±1.04	102.3^{b}±1.00	95.2^{b}±0.64	NS
DAY 14	101.27^{c}±1.11	90.3^{a}±1.52	98.3^{b}±1.52	2.28
DAY 21	102.94^{c}±.91	88.44^{a}±1.38	91.16^{b}±1.25	2.42

Values are expressed in Mean±SD (Standard Deviation), ****** Significant at p≤0.05

NS-Not significant

Means within rows separated by Duncan's multiple range test p=0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I - T_4 + *Lactobacillus plantarum,* CFM II - T_6 + *Lactobacillus casei*

It was observed from the above data that the blood glucose levels of the animals fed with normal rat ration (Group I) and the blood glucose levels of the animals fed with the formulated test diets (Group II and III) did not differ significantly (p≤0.05) until 7^{th} day of the experiment. It can be concluded that formulated mixes had no effect on the fasting blood glucose levels of the experimental animals on day 0 to day 7. However, on day 14 and 21, the blood glucose levels of the experimental groups were statistically different at (p≤0.05) from each other. The decrease in the blood glucose level was more in group II followed by group III.

Findings of the present study were similar with the work done by Narayanan *et al.,* (2016) who reported that supplementation of a meal with lower GI foods like finger millet and flaxseed resulted in the reduction of glycemic profile of experimental animals.The high dietary fibre content has been found to reduce gastric emptying and absorption of glucose after a meal, resulting in improved glucose tolerance (Bahadoran *et al.,* 2013). The role of soluble dietary fibre decreases the activity of digestive enzymes, resulting in incomplete hydrolysis of carbohydrates, protein, fats and delays absorption (Kam *et al.,* 2016).

Kelley *et al.,* (2003) reported that non digestive fibre or crude fibre in functional ingredients like millets and legumes are fermented by the microflora of the colon resulting in the production of short chain fatty acids which may slow

down carbohydrate metabolism and decreasing serum free fatty acids which reduce blood glucose levels through competition in insulin-sensitive tissues. Several studies have also suggested that rice, finger millet and pumpkin seeds contains resistant starch or slowly digestible starches which have a number of potential mechanisms in lowering blood glucose levels (Kam *et al.,* 2016). Hence the functional ingredients used in the present study were in accordance with the findings mentioned above.

Hence, it was recommended that after 7th day of feeding period, the blood glucose levels in the developed probiotic complementary food mixes CFM I and CFM II was statistically different from the rat ration.

The total blood protein levels of the Wistar albino rats are presented in Table 4.26.

Table 4.26. Total blood protein levels in Wistar albino rats

	Group I	Group II	Group III	CD 0.05
DAY 0	8.99^{a}±0.02	10.75^{b}±0.25	9.51^{a}±0.47	NS
DAY 7	9.81^{a}±0.69	11.50^{b}±0.49	10.53^{a}±0.45	NS
DAY 14	9.59^{a}±0.40	13.03^{b}±0.44	13.04^{b}±0.43	0.05
DAY 21	7.35^{a}±0.39	12.51^{b}±0.48	13.29^{b}±0.61	0.01

Values are expressed in Mean±SD (Standard Deviation), ****** Significant at p≤0.01
NS-Not significant

Means within rows separated by Duncan's multiple range test p=0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

From the above table, it can be concluded that the total blood protein levels of the animals fed with normal rat ration (Group I) and the total blood protein levels of the animals fed with the developed probiotic complementary food mixes (CFM I and CFM II) did not differ significantly (p≤0.05) until the 7th day of the experiment. On day 14, the total blood protein levels of the experimental groups were statistically different at (p≤0.01) from each other.

PHASE VI

4.7. Shelf life studies of the developed probiotic complementary food mixes

The shelf life studies of the developed probiotic complementary food mixes were done by storing the mixes at ambient temperature (25-30°C).

4.7.1. Free fatty acid of developed probiotic complementary food mixeson storage

The mean free fatty acid content of the formulations on storage periods are presented in Table 4.27.

Table 4.27. Free fatty acid of developed probiotic complementary food mixeson storage

Parameters	Period of evaluation (Days)	CFM I	CFM II	CD 0.05
Free fatty acid (%)	**0**	1.06±0.03	1.71±0.06	0.04**
	30	3.17±0.28	2.78±0.07	0.04**
	60	3.98±0.04	3.20±0.05	0.05**
	90	4.29±0.05	4.05±0.05	0.02**

Values are expressed in Mean±SD (Standard Deviation), ****** Significant at p≤0.05

Means within rows separated by Duncan's multiple range test p=0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

The free fatty acid values at day zero for CFM I was 1.06±0.03% for CFM II was 1.71±0.06%,at 30 days for CFM I was 3.17±0.28%for CFM II was 2.78±0.07%, at 60 days for CFM I was 3.98±0.04% for CFM II was 3.20±0.05%, at 90 days for CFM I 4.29±0.05% was for CFM II was 4.05±0.05%. It has been inferred from the above data that there was a significant increase (p≤0.05) in the mean free fatty acid values of both the formulation CFM I and CFM II on storage.

The difference in the free fatty acid content between both the formulations may be due to differences in the raw ingredients used for the preparation of the

food mixes. The increase may be due to the higher moisture content, effect of temperature and presence of bran in the rice samples. Increase in total amount of free fatty acid during storage of mixes might be attributed to the activities of lipases and lipolytic acyl-hydrolases (Molteberg *et al.,* 1995).

Ahmed (2014) also reported increase in mean free fatty acid of complementary formulations prepared from rice, malted green gram, ground nut and sesame. Similarly Saeeda*et al.,* (2009) formulated a nutritious instant food mix and found free fatty acid content of 2.43-3.65g/100g during 45 days of storageSimilarly, Salve *et al.,* (2011) in his study of supplementary foods formulated from flours of wheat, soybean and chick pea found that during storage of 90 days there was a gradual increase in the fatty acid contents in all the blends. However, the average value of free fatty acid content in his study (0.115 to 0.201 mg /100g) was lower than the present study which may be due to the storage of the blends.The conclusions in the above mentioned studies were in accordance to the present study where the CFM I, which was rice-based, had free fatty acid values significantly higher than CFM II.

The free fatty acid was within the permissible limit by Codex Alimentarius, 1992 i.e. 4g/100g of sample (Odoom *et al.,* 2014). Hence, it can be concluded from the above study that both the developed formulations can be stored for a period of 60 days at ambient temperature (20-25°C). No remarkable change was observed up to 60 days of storage which may be due to the effect of drying temperature, packing of the flours in polyethylene bags, sealed and stored in ambient temperature.

4.7.2. Peroxide values of developed probiotic complementary food mixeson storage

The mean peroxide values of formulated complementary food mixes are presented in Table 4.28.

Table 4.28. Peroxide values of developed probiotic complementary food mixeson storage

Parameters	Period of evaluation (Days)	CFM I	CFM II	CD 0.05
Peroxide (m moles/kg fat)	0	3.05±0.05	4.10±0.09	0.00**
	30	4.10±0.09	5.03±0.05	0.01**
	60	5.18±0.07	6.18±0.03	0.00**
	90	6.95±0.05	7.05±0.04	0.03**

Values are expressed in Mean±SD (Standard Deviation), ****** Significant at p≤0.05

Means within rows separated by Duncan's multiple range test p=0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

The peroxide values at day zero for CFM I was 3.05±0.05 mmoles/kg fat andfor CFM II was 4.10±0.09 mmoles/kg fat. At 30 days for CFM I it was 4.10±0.09 mmoles/kg fatfor CFM II was 5.03±0.05 mmoles/kg fat, at 60 days for CFM I was 5.18±0.07 mmoles/kg fatfor CFM II was 6.18±0.03 mmoles/kg fat, at 90 days for CFM I 6.95±0.05 mmoles/kg fat was for CFM II was 7.05±0.0 mmoles/kg fat. The difference in peroxide values of the formulations were significant (p≤0.05) on storage periods and there was a significant increase (p≤0.05) in the mean peroxide values across storage.

The results of the present study were in accordance to Lohia and Udipi (2015) who developed a complementary food from wheat, ragi, lentil and amaranth and reported peroxide value of 5.12 mEq/kg which increased to 9.94 mEq/kg after 14 days of storage. This short shelf life may be due to storage in polyethylene bags at ambient temperature. However, Ahmed *et al*., (2008) in his study of complementary mixes made from wheat flour and soya flour in different proportions reported peroxide values (8.27% to 8.67%) which were similar to the present study but no significant changes were observedin his study up to 4 months of storage which may be due to the use of sealed polyethylene bags and storing in ambient temperature.

The packaging material considered suitable for the storage was polyethylene virgin containers as quoted by Raza *et al.,* (2009) in his study. He formulated a nutritious instant baby food from 50% cereals, 30% legumes, 10% vegetables and 10% nuts and seeds and found significant increase in the peroxide content (8.83 to 8.62 mmoles per kg of fat) during 3 months of storage in double polyethylene bags at ambient temperature(20-25°C).

The findings of the present study were within the standard specified by Prevention of Food Adulteration Rule, 1991 (10 mmoles/kg fat). Therefore, the formulated mixes CFM I and CFM II could be considered acceptable in all the storage period as it was below the recommended value. The difference in the peroxide content between the formulations may be due to differences in the rice varieties used for the preparation of the food mixes. The increase in peroxide values during storage is probably due to peroxidation of double bonds in unsaturated fatty acids which respectively breakdown in order to produce secondary oxidation products that may indicate rancidity (Gahlawat and Sehgal, 1994).

4.7.3. Viable cell count of probiotic complementary food mixesCFM I and CFM II on storage

The viable cell count of CFM I and CFM II on storage are presented in Table 4.29.

Table 4.29. Viable cell count of probiotic complementary food mixesCFM I and CFM II on storage

Days	CFM I (cfu/g)	pH	CFM II (cfu/g)	pH
0th day	6.2×10^{8}	5.9	7.0×10^{8}	6.1
7th day	6.0×10^{8}	5.8	6.7×10^{8}	5.9
14th day	5.8×10^{8}	5.8	6.5×10^{8}	5.9
21th day	5.5×10^{8}	5.7	6.3×10^{8}	5.8
28th day	5.1×10^{8}	5.6	5.8×10^{8}	5.7
45th day	5.0×10^{8}	5.6	5.4×10^{8}	5.6
60th day	4.7×10^{8}	5.5	5.1×10^{8}	5.6

Values are Mean±SD of 3 replications

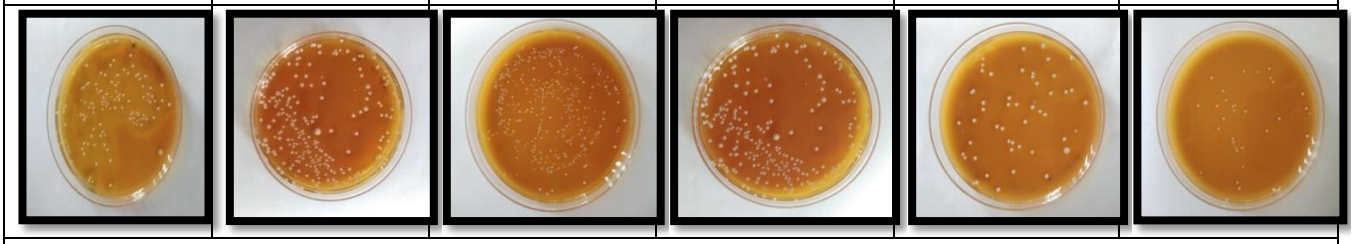
Means with different superscript within the same row are significantly different at p≤0.05

The most important factor for these probiotificated products is the microorganisms viability, which is measured as the number of live and active cells present in per g or ml of probiotic food items at the time of consumption. This value defines how efficient and useful these products are for consumers (Dharmasena, 2012).Plate 28 has shown the bacterial growth up to 60^{th} day of storage study.

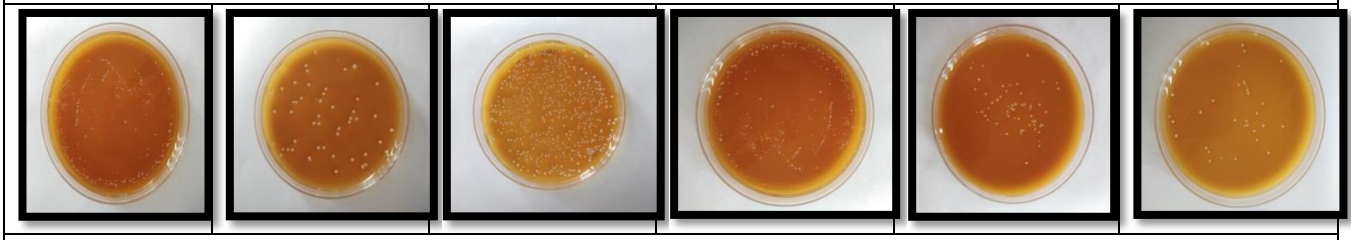
Both the formulations CFM I and CFM II contained 10^8cfu of viable probiotic cells per ml/g of sample on storage for 60 days at ambient temperature of 20-25°C and fulfilled the FSSAI (2016) requirements, to be claimed as a probiotic food mix.In 2016 Food Safety and Standards Authority of India (FSSAI) under section 16(5) recommended that a product is referred to as a probiotic food if it contains maximum 10^6 to 10^8cfu of viable probiotic cells per ml/g of sample at the time of consumption.

Therefore, it is important to ensure a high survival rate of probiotic bacteria during production as well as during storage.The pH of probiotic products considerably affects cell survival of probiotic microorganisms (Nematollahi*et al.,* 2016).The growth and stability of the probiotic strains are restricted when the pH is very low or high in probiotificated mixes or products. Strains can survive pH of about 5.5 to 6.0 in probiotified products (De Vuyst and Degeest, 2000). Hence in the present study we can observe, increase in pH values with increase in storage time but the pH levels were within the permissible limit of 5.5 to 6.0 as mentioned above.

From the above findings it can be inferred that both the probiotic complementary food mixes CFM I and CFM II had the required amount of viable probiotic count as per FSSAI requirements mentioned above.



Rice based probiotic complementary food mix (CFM I)



Finger millet based probiotic complementary food mix (CFM II)

Plate 28. Total bacterial growth up to 60th day of storage study

4.7.4. Fungal count of probiotic complementary food mixes CFM I and CFM II on storage

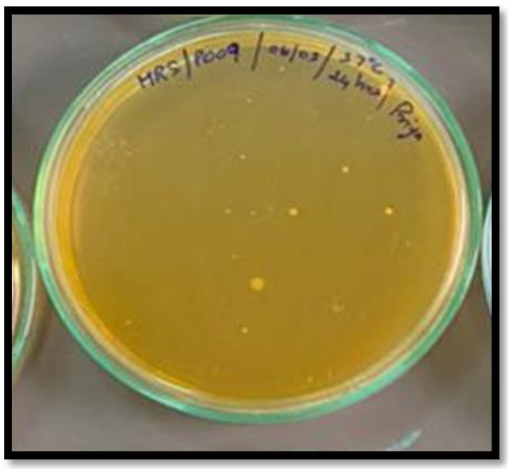
The fungal count of probiotic complementary food mixes CFM I and CFM II on storage is shown in Table 4.30.

Table 4.30. Fungal count of probiotic complementary food mixes CFM I and CFM II on storage

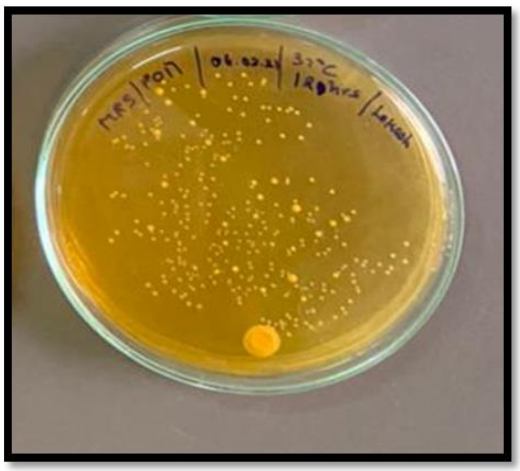
Days	CFM I (cfu/g)	CFM II (Cfu/g)
0th day	Nil	Nil
7th day	Nil	Nil
14th day	Nil	Nil
21th day	Nil	Nil
28th day	Nil	Nil
45th day	Nil	Nil
60th day	Nil	Nil
75th day	0.3 x10^{1}	0.4 x10^{1}

The study on the fungal count of probiotic complementary food mixes CFM I and CFM II on storage at ambient temperature revealed that till the 60th day, total fungal count were below the detectable level for the developed probiotic complementary food mixes CFM I and CFM II. However on the 75th day of storage, 0.3 x10^{1} cfu/g was observed in CFM I and 0.4 x10^{1} cfu/g was observed in CFM II respectively. Plate 29 has shown the fungal growth on the 75th day of storage study.

Hence, it can be reported from the above study that the probiotic complementary food mixes can be stored for a period of 60 days without any fungal growth.Therefore, the null hypothesis 'H_0 -The probiotic complementary food mixes cannot be significantly stored for more than 90 days at room temperature' was rejected.



Rice based probiotic complementary food mix (CFM I)



Finger millet based probiotic complementary food mix (CFM II)

Plate 29. Total fungal growth after day 75 of storage study

4.7.5. Effect of metallized polyethylene packaging on moisture content of complementary food mixes

Data pertaining to the effect of storage period on moisture content of the complementary food mixes for a period of 90 days are presented in Table 4.31.

Table 4.31. Effect of metallized polyethylene packaging on moisture content of developed probiotic complementary food mixes

Formulations	Moisture content (%)						
	0 days	15 days	30 days	45 days	60 days	75 days	90 days
CFM I	3.23±0.30^{NS}	3.30±0.05^{NS}	3.35±0.30^{NS}	3.41±0.50**	3.60±0.10**	3.65±0.20**	4.01±0.10**
CFM II	3.84±0.15^{NS}	3.90±0.20^{NS}	3.93±0.30^{NS}	3.95±0.10**	4.00±0.20**	4.01±0.30**	4.05±0.50**

Values are expressed in Mean±SD (Standard Deviation), ****** Significant at p≤0.01

NS-Not significant

Means within rows separated by Duncan's multiple range test p=0.01

Means followed by the same letter shown in superscript(s) are not significantly different

CFM I- T_4 + *Lactobacillus plantarum,* CFM II- T_6 + *Lactobacillus casei*

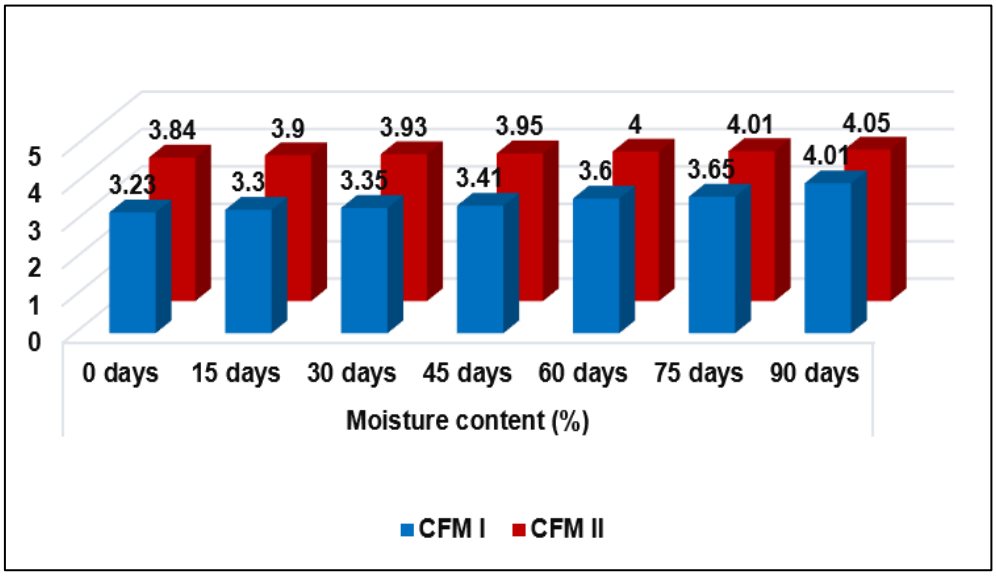


Fig. 4.13. Moisture content of developed probiotic complementary food mixes at day 90 of storage at ambient conditions

The probiotic complementary food mixes were stored in sealed metallized polyethylene pouches at ambient temperature of 20-25°C. With the progression of storage period, there was significant increase in moisture content after 45 days. It was observed that there was no significant increase in the moisture content up to 30 days but after 30 days there was significant increase in moisture content. Increase in moisture content will adversely affect the shelf life of probiotic complementary food mixes. This indicates the limitations of packaging material.

4.8. Cost estimation for the development of probiotic complementary food mixesin comparison to the commercial formula

The cost estimation for the development of probiotic complementary food mixes in comparison to the commercial formula is presented in Table 4.32.

Table 4.32. Cost estimation for the development of probiotic complementary food mixes in comparison to the commercial formula

Commercial complementary formula	Cost/kg (Rs.)	Developed probiotic complementary food mixes	Cost/kg (Rs.)
Commercial formula I (Soy based formula)	1166.6	CFM I	179
Commercial formula II (Whey protein based formula)	750	CFM II	180
Commercial formula III (Rice based formula)	1237.5	-	-

The cost of both the developed probiotic complementary mixes CFM I and CFM II in the present study ranged from Rs. 179 to Rs. 180 per kg. It was evident that boththe complementary food mixes were less costly than commercial infant formula. Cost of the three renowned commercial complementary foods ranged from Rs. 750 per kg to Rs. 1,237.50 per kg. One of the factors limiting the use of commercial infant formula is their high cost and affordability by the common man.

The cost of the developed probiotic complementary food mixes CFM I and CFMII were calculated according to the prices prevailing for the raw ingredients in Coimbatore city. These developed complementary food mixes may be agood alternative for low income familiesto provide nutrient rich formula with less viscosity as probiotic complementary food.

From the foregoing results, it is revealed that it was feasible to develop probiotificated complementary food mixes using low cost locally available

cereals/millets, legumes and oil seeds using four different strains namely *Lactobacillus fermentum*, *Lactobacillus casei, Lactobacillus rhamnosus,* and *Lactobacillus plantarum* respectively. Further, the probiotified complementary food mixes were found to be rich in macro and micronutrients and possess high energy density potentials. The physical properties after malting, fermentation and probiotification improved the overall acceptability and shelf life of the products. Both the products when kept at ambient temperature was found to be stable for a period of 60 days, and the peroxide values, free fatty acid content and viable count of microorganisms were within the permissible limit. The probiotic complementary food mixes on feeding to Wistar strain albino rats showed improvement in the diarrhoeal induced groups along with it the microorganisms present were able to survive the gastrointestinal transit increasing the useful bacteria in the fecal count. The body weight, blood glucose and protein of Wistar albino rats showed significant increase during the feeding trials. Further studies are recommended for infants to study in depth effectiveness of the developed mixes in human models.