CHAPTER II

REVIEW OF LITERATURE

The review of literature pertaining to the present study on "**Assessment of nutritional and functional properties of probiotic complementary food mixes from locally available cereals and legumes**" are discussed under the following heads:

2.1. Global scenario and importance of used cereals/millets, legumes and oil seeds

2.2 Concept of complementary food mixes

2.2.1. Complementary foods and feeding

2.2.2. Processing techniques used in complementary foods

2.2.3. Nutritional analysis of complementary foods

2.2.4. Functional properties of complementary foods

2.2.5. Storage stability of complementary foods

2.3.Concept, definition and historical perspective of probiotics

2.3.1. Health benefits of probiotics

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2.5.1. Freeze drying

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2.1. Global scenario and importance of used cereals/millets, legumes and oil seeds

The gap between world human population and quantity of food supply has already become a global treat.Therefore, to bridge the gap, efforts are being made to identify and evaluate underutilized food sources. Agriculture is under increasing pressure to produce greater quantities of food, feed and biofuel on limited land resources for the projected nine billion people on the planet by 2050 (FAO, 2010). Many studies reported that cereals/millets and legumes are the powerhouse of phytonutrients and phytochemicals including enzyme inhibitors, phyto-hemagglutinnins (lecitins), phytoestrogens, oligosaccharides, saponins and phynolic compounds, which play metabolic role by providing numerous health benefits in protecting against coronary heart diseases, diabetes, high blood pressure and inflammations (Arisa and Aworh, 2007; Doss *et al.* 2011; Bouchenak and Senhadji, 2013).

Cereals are synonyms of Indian food production, obviously due to its lion share (~ 90 %) in total Indian food basket. Since time immemorial, fate of Indian agriculture heavily depends upon the success of cereals production. Cereals are the leaders in the food commodity export especially rice.

Most common cereal available all over the world and today is even more in demand for its abundant health benefits is rice. Through decades, rice has been one of the major cereals crops in the world. FAO (2021) estimated production of 520.8 million tones of rice which accounted for half the global production of primary crops along with three other crops (https://www.fao.org/worldfoodsituation/csdb/en/). The total food grain production is estimated at 305.44 million tones out of which rice production is 121.46 million tones. Fig. 2.1.shows the highest rice producing Asian countries in the year 2020, according

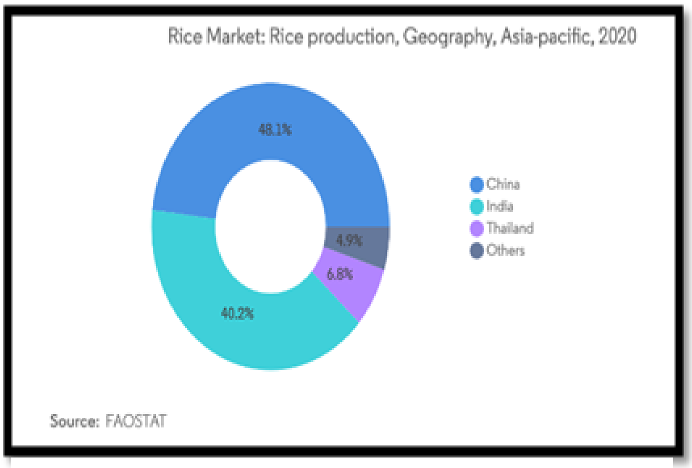
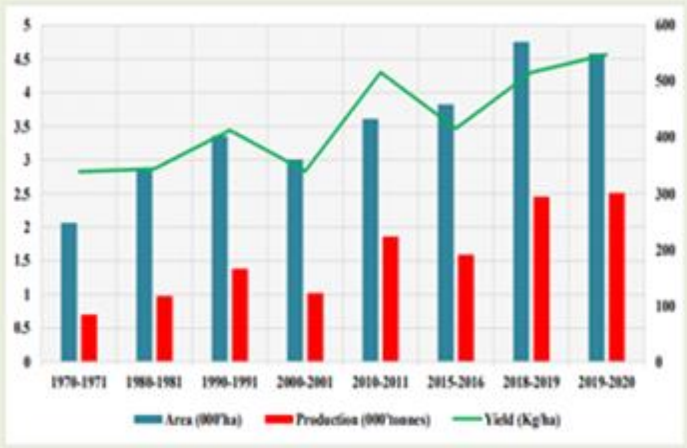


Fig.2.1. Highest rice producing Asian countries,

to recent data given by FAOSTAT, 2020 (https://www.fao.org/faostat/en/#home). Rice is a major cereal food crop and staple food in most of the developing countries. India stands second in the production of rice next to China (Rathna *et al.,* 2019). Rice flour is one of the main foods consumed by most Asian countries especially India. Rice is a staple food of the world and being a major source of carbohydrates over half the world's population.

Millets are known as ancient nutritional grain and important food staples, particularly, in poor, semi-arid tropics of Asia and Africa (Mahendra, 2012 and Narloch *et al*., 2009) which are mostly cultivated under a variety of agro-ecological situations like plains, coast hills even diverse soil land varying rainfall. Millets are most popular in developing regions, like India and Africa, where food and nutritional security are the major challenges. According to Food and Agriculture organization 2019, the global millet production was estimated at 28.2 million metric tons in 2019, which had increased to 30.5 million metric tons in 2020. India is the largest global producer, with a 33.3% global market share in 2020 (https://www.fao.org/worldfoodsituation/csdb/en/). It is estimated that the share of the global finger millet production area is about 12.5% of the millet (Gebreyohannes *et al.,* 2021). India is the leading producer of various millets, finger millet ranks fourth on a global scale of production next to sorghum (*Sorghum bicolor*), pearl millet (*Cenchrus americanus*) and foxtail millet (*Setariaitalica*) (Maharajan *et al.,* 2021). Fig.2.2. shows millet map of India, data given by Ministry of Agriculture and Farmers welfare, 2022 (https://agricoop.

FIG. 2.2.MILLET MAP OF INDIA. MINISTRY OF AGRICULTURE & FARMERS WELFARE, 2022



Source: www.indiastat.com

FIG.2.4. AREA, PRODUCTION AND YIELD OF MUNGBEAN IN INDIA

nic.in/en). The International year of millets 2022-23 was announced in India to encourage the Post-harvest value addition of millets, increasing local consumption and branding millet commodities domestically and globally.United Nations declared 2016 as International Year of Pulses to heighten people awareness of the nutritional benefits as part of sustainable food production (improving soil fertility and environment) aimed towards achieving food and nutritional security. Presently, at the global level, pulses are the second most important group of crops after cereals. Pulses are produced on 12-15 percent of global arable land and contributing 33 percent of the dietary protein nitrogen (N) needs of humans (Morel *et al.,* 2012). The global pulses production was 86.32 million tonnes from an area of 88.47 million ha with an average yield of 975 kg per ha in 2018 (FAO, 2018). The major contribution in global pulses production was Asia (43.55 percent).Pulses are the backbone of Indian agriculture as well as the predominantly vegetarian diets of millions of people. Pulses are consumed equally by India's rich and poor as it is one of the less expensive sources of protein (Mohanty and Satyasai, 2015). Fig. 2.3.depicts area and production of pulses in India from 1950 to 2019 the data was collected from Farmers portal (https://farmer.gov.in/cropstaticspulses.aspx). Pulses on account of their vital role in the nutritional security and soil ameliotive properties have been an integral part of sustainable agriculture since ages. Pulses, as an important source of protein, constitute a basic ingredient in the diet of vast majority of poor and vegetarian population in India. Pulses are not only a low cost source of protein for majority of Indian consumers but also low cost substitute for vegetables in periods of their higher prices. India is largest producer of chickpea, mung bean and lentil are important pulses contributing 43.29 percent, 10.03 percent and 6.67 percent, respectively, to the total pulses production (23.40 million tonne) in India (FAO, 2018). Green gram scientifically known as *Vigna radiata* is a plant species in the legume family and commonly called as mung bean, moong in India. India is its primary origin and is mainly cultivated in East Asia, Southeast Asia and the Indian subcontinent. It is the third important pulse crop of India grown in nearly 16 per cent of the total pulse area of the country. It contains proteinrich seed with 20-25 % protein and sometimes plants are cut and ploughed into the soil to enrich soil nitrogen. India is the major producer of green gram in the world and grown in almost all the States. It is grown in about 4.5 million hectares with the total production of 2.5 million tonnes with a productivity of 548 kg/ha and contributing 10 % to the total pulse production. According to Government of India 3rd advance estimates (Kumar, 2022), green gram production in 2020-21 is at 2.64 million tonnes. Fig. 2.4 shows area, production and yields of mung bean in India from the year 1970 to 2020. The data was taken from greengram outlook report, January-March 2021, and retrieved from the website of https://angrau.ac.in/ downloads /AMIC/ GREENGRAM/202021.pdf.

FIG. 2.5. TOTAL SESAME SEEDS PRODUCING COUNTRIES IN TONNES

India is one of the major producers of many oilseed crops like groundnut, mustard, rapeseed, sesame seed, etc. Traditionally, Indians consume substantial quantity of edible oils mainly as a cooking medium. Among the oilseed crops, sesame has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein. Sesame (*Sesamum indicum L.*) is one of the world's important oil crops. Its primary marketable products are the whole seeds, seed oil and meal. Sesame seeds were one of the first crops processed for oil as well as one of the earliest condiments (de Carvalho *et al.,* 2001). It is rich in unsaturated fatty acids where the fatty acids composition is 14% saturated, 39% mono-unsaturated, and 46% poly-unsaturated fatty acids (Toma and Tabekhia, 1979). Carbohydrates in sesame seed are composed of 3.2% glucose, 2.6% fructose and 0.2% sucrose while the remaining quantity is dietary fibers. Fig. 2.5.shows total sesame seeds producing countries in tones in the year 2018 (https://www.tridge.com/intelligences/sesame-seed/production).

The pumpkin seeds (*Cucurbita sp.*) from Cucurbitaceae family are usually considered as industrial waste products and thrown out. In some area's seeds are utilized as uncooked, cooked or roasted, although simply for the domestic purpose. As they are rich in protein, fibers, minerals like iron, zinc, calcium, magnesium, manganese, copper and sodium, PUFA (polyunsaturated fatty acids), phytosterol and vitamins, they might be considered important for the food industries. Fig. 2.6.shows world top 10 pumpkin producing countries (https://worldmapper.org/maps/pumpkin-production). As the seeds are considered as by-product of the pumpkin fruit, they are cheaper in cost and their utilization is different food products may lead to enhance their nutritional value at lower cost. Health promoting impacts of pumpkin seeds on the level of blood glucose, cholesterol, immunity, liver functioning, gallbladder, prostate gland, depression, inflammation, cancer management and inhibition of parasites are established. These are the factors causing rapid market growth for pumpkin seeds in the extract, oil, raw seeds or roasted form. It has been a popular snack since the World Health Organization in 2003 (WHO,2003) declared it to be the greatest food to eat for obtaining zinc and magnesium.

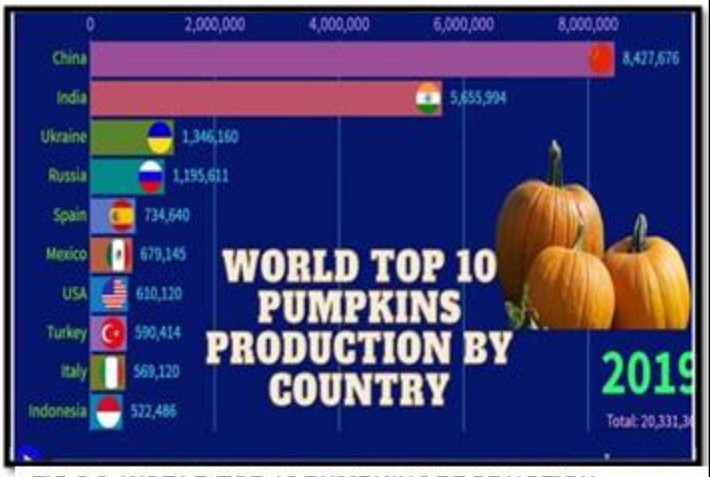


FIG.2.6. WORLD TOP 10 PUMPKINS PRODUCTION

2.2 Concept of Complementary food mixes

2.2.1. Complementary foods and feeding

Complementary foods in liquid, semi-solid and solid form are developed for infant to provide required nutrients adequately. Complementary feeding is the process that starts when breast milk is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods are needed in addition to breast milk. The term ‘weaning foods’ are now-a-days no longer recommended to use as a synonym for ‘complementary food’ because the term ‘weaning’ means total cessation of breastfeeding. In order to have optimal growth, development and health, infants should be breastfed for the first six months of life. Thereafter, transition from only breast-milk to other solid and semisolid foods, along with breast-feeding is referred as complementary feeding which covers 6- 24 months of age (WHO, 2000; WHO, 2003; Sajilata *et al*., 2002; Alexander, 1983; Lutter and Rivera, 2003; Prentice and Moore, 2005).

Processed-cereal based complementary food should contain milled cereal and legumes combined not less than 75 per cent and the product is intended to be mixed with water or milk before consumption. All ingredients, including optional ingredients, shall be clean, safe, suitable and of good quality. It may also contain following ingredients - protein concentrates, essential amino acids, iodized salt; milk and milk products; eggs; edible vegetable oils and fats, fruits and vegetables; various carbohydrates such as sucrose, dextrose, dextrin, and carbohydrate rich foods like honey, corn-syrup, malt and potatoes. The material shall be manufactured and packed under hygienic conditions (Codex, 1994; BIS, 2006).

The ideal time to introduce complementary foods in the diets of infants is difficult to pinpoint. Complementary foods introduced too early are of little benefit to the infant and may even be harmful due to the possibility of choking, developing food allergies, or causing an infant to consume less than the appropriate amount of breast milk or infant formula. Introducing complementary foods too late may cause an infant to develop nutritional deficiencies and/or miss that period of developmental readiness. Consequently, the infant may have difficulties learning to eat complementary foods when they are introduced later. When complementary foods are introduced appropriate to the developmental stage of the infant, nutritional requirements can be met and eating and self-feeding skills can develop properly. Paediatric nutrition authorities agree that complementary foods should not be introduced to infants before they are developmentally ready for them; this readiness occurs in most infants between 4 and 6 months of age (USDA, 2009).

Protein energy malnutrition is an important nutritional deficiency condition that often occurs during the critical transitional phase of weaning infants, crippling their physical and mental growth. This could be due to progressive decline in the incidence of breast feeding observed during the last 25-30 years (Acharya and Shah, 1998). This condition can be prevented to a large extent by introducing weaning foods at right proportion and at right stage (Pawar and Dhanvijay, 2007).

The prominent brands of commercial weaning foods offer excellent nutrition for babies but are expensive which cannot cater to the needs of babies belonging to the low socio-economic status. The formulation and development of weaning foods from locally and readily available raw materials has received a lot of attention in many developing countries (Ijarotimi and Aroge, 2005).

2.2.2. Processing techniques used in complementary foods

For commercial preparation of weaning food, the most commonly adopted methods are roller drying, extrusion cooking and spray drying to a limited extent. However, weaning food can also be made based on some traditional food processing techniques such as malting or sprouting of cereals and legumes, fermentation, popping and flaking cereals (Malleshi, 1995; Wambugu, 1996).

Gahlawat and Sehgal (1994) observed that starch and protein digestibility and iron bioavailability of weaning foods was improved by using roasting and malting techniques to process cereals and pulses for development of weaning foods. Wondimu and Malleshi (1996) developed weaning foods based on barley and chickpea by adopting malting techniques. The protein quality of the formulation was improved significantly. Germination converts insoluble protein to soluble compounds and increases the level of lysine as well as of vitamin B and C (Brandtzaeg *et al.*, 1981; Mbofung and Ndjouenkun, 1990;). Wang and Field (1978) revealed that germination increased the level of lysine and methionine in sorghum. Kulkarni *et al*. (1991) claimed that malted weaning foods had high in vitro digestibility and a fair amount of available lysine (3.24 -4.17g/16 g N), and

PER in the range of 2.78 to 2.82. Weaning formulas based on malted sorghum and cowpeas were nutritionally superior to unmalted roller-dried one. They had considerably lower paste viscosity, significantly higher PER (2.26) and available lysine (3.85%) than roller-dried food (Malleshi *et al*., 1989; Salunkhe *et al*., 1992).

Processing techniques are known to improve nutritional profile of weaning food by decreasing anti-nutritional components. Food processing methods to remove or degrade phytate have been recommended in order to improve iron absorption (Hurrell, 2004). Some of the phytates are water soluble in nature and can be reduced by soaking cereal in water (Perlas and Gibson, 2005). Bioavailability of iron increased by 62 per cent in green gram, 39 per cent in chickpea and 20 per cent in finger millet with a reduction in tannin content upon germination (Hemalatha and Srinivasan, 2007). Low cost, nutritious and highly digestible weaning food was developed by using malted ragi and malted green gram (Malleshi and Desikachar, 1982). This enhanced the nutritive value of foods by increasing lysine and tryptophan and reducing anti-nutritional factors and improved the overall bioavailability of nutrients (Kadam *et al.,*1984).

Obizoba (1990) and Villages *et al.,*(1968) in a study of nutritional evaluation of blends of corn with germinated cowpea, pigeon pea, and bambara-groundnut, concluded that germinated corn with germinated legumes showed nutritional superiority to ungerminated blends. However, recently there has been growing interest in the application of malting to improve the acceptability and nutritional value of locally based weaning foods in many developing countries (Kulkarni, and Ingle,1991).

Plahar *et al*., (2003) standardized an extrusion cooking process for production of high protein weaning food based on peanuts, maize and soybeans. Sadana and Chabra (2004) developed low cost weaning foods namely *panjiri, kheer, halwa* and *dalia* using germination, malting, roasting and pressure cooking processes. The experimental formulations were based on germinated wheat, pulses (Bengal gram, green gram and lentil) and roasted groundnut in the ratio of 75:25:25. The grains were allowed to germinate at room temperature in wet muslin cloth for 24-48 hours. Germinated grains were dried at 60°C for 7-8 hours.

Germinated and un-germinated dried grains were milled into flour and *dalia*. The germinated grain flours were used to develop experimental formulations while ungerminated wheat formulations were treated as control.

Fagbemi *et al.,* (2005) reported that processing significantly reduced antinutritional factors of fluted pumpkin seed. Total phenols of *curcas* seeds significantly reduced ($p<0.05$) due to roasting at 160ºC for 30 minutes. Suitable and effective processing of the complementary foods and weaning foods is an important step to not only to improve nutritional and functional properties of the developed food but also to improve the shelf life (Ikujenlola and Fashakin, 2005).

Roasting is one of the processing steps to improve the flavour of grains.. Roasting of cereals, pulses and oilseeds is a simple and common household and village level technology which is reported to remove most anti-nutritional factors such as trypsin inhibitor, hemagglutinin, gioterogenic agents, cyanogenic glycosides, alkaloids and saponins and increases storage life (Thapaliyal and Singh, 2015).

2.2.3. Nutritional analysis of complementary foods

The nutritional value of two weaning mixes developed using pearl millet or barley with roasted amaranth, green gram and jaggery in the ratio of 60:20:40:45 revealed that the moisture, protein, energy, ash, iron and calcium contents of the mixtures ranged from 5.90 to 6.03g, 9.84 to 9.95g, 416 to 441 Kcal, 3.77 to 4.32g, 17.75 to 19.42 mg and 150 to 190 mg per 100g respectively (Gupta and Sehgal, 1991). Mixes developed using sorghum, green gram and sesame flour at different proportions (Kulkarni*et al.,*1991) contained 14.31 g to 17.46 g of protein, 3.10 to 3.40/ 16g N available lysine 2.35-2.63 g ash, 1.7-2.34 g crude fiber, 75.5-80.83 g carbohydrates, 361 -385 Kcal, 18.29-26.2 mg ascorbic acid and 11.37-12.01 mg of iron. The formulated composite mix using maize (20%), sweet potato (50%), soybean (25%) and peanut (5%) contained 14-25 per cent protein, 6.41 per cent fat, 2.39 per cent ash, 67.36 per cent carbohydrates, 59.6 mg calcium, 187.5 mg phosphorous and 2.4mg iron per 100g (Idowu *et al*., 1993). Kshirsagar *et al*.,(1994) formulated and nutritionally tested four weaning foods based on finger millet, green gram, peanut, skim milk powder in the ratio 35:35:10:20 with or without addition of 5 per cent barley malt. Protein content of the mixes ranged from 20.8-21.7 per cent, fat from 3.5 -3.7 per cent, ash from 3.1-3.7 per cent and crude fibre 1.64-1.79 per cent.

Geetha and Suja (1996) formulated two mixes using rice or finger millet, lima bean and peanut at the ratio of 65:25:15 and analysed the nutrient composition. The authors reported that every 100g of finger millet based mix had higher protein (12.08g), fat (6.8g), ash (1.10g), iron (2.5mg), phosphorus (283mg) and calcium (260mg) compared to rice based mix (12.20g, 5.9g, 0.9g, 0.5mg, 205mg and 63mg/100g respectively). Roasted and malted weaning mixes using amaranth grain and Italian millet in the ratio of 70:30 were developed by Suma (1998) reported that the roasted mix contained higher protein (14.79g/100g), fat (4.75g/100g) and ash (2.24g/100g); lower fibre (1.73g/100g) and carbohydrates (71.79g/100g), compared to malted mix (14.15g, 4.15g, 2.21g, 2.05 and72.16g per 100g of mix respectively).

Banakar (2005) developed supplementary foods using roasted or malted sorghum, finger millet, green gram and roasted rice, soybean and peanuts. To enhance the micro nutrients powdered amaranth leaves were added. When analysed for nutritional quality, roasted food contained significantly higher amount of protein (16.88%), fat (4.27%) and ash (2.97%) compared to malted (15.96, 3.89 and 2.86% respectively) while, malted food contained significantly higher amounts of moisture (5.93%), crude fibre (2.52%) and total carbohydrates (68.84%) compared to that of roasted (5.89, 2.43 and 67.56% respectively). The energy value of roasted and malted foods was 376 and 374 Kcal respectively. Every 100 gram of malted mix had comparatively higher amounts of calcium (430.50 mg), iron (11.18mg), zinc (4.5mg) and copper (3.48mg) than roasted (4.27, 10.97, 4.28 and 3.39mg respectively).

Ahmed *et al.,* (2008) formulated six mixes using flours of wheat and soybean at different proportions. Soybean was heat treated for 5, 10 and 15 min, before powdering and mixed with wheat flour at the ratio of 95:5 or 90:10. Further 3g of milk powder and 5g sugar were blended to all the mixes and analysed for proximates. Mixes contained 12.52 to 13.63g protein, 4.58 to 4.88g fat, 1.47 to 1.57g ash and 72.69 to 73.72g carbohydrates.

In a study by Satter *et al.,*(2013) and Imitiaz *et al.,* (2011) there was an attempt to develop nutritionally enriched instant weaning food and evaluate its safety aspects. The developed instant weaning food contained the major nutrients like moisture, ash, fat, protein, fibre, carbohydrate and energy 2.43 per cent, 2.26 per cent, 11.32 per cent, 15.98 per cent, 1.06 per cent, 75.35 per cent and 456.6 kcal/ 100 g, respectively and was comparable to three good quality imported commercial weaning foods.

Malleshi (1995) stated that weaning foods are modification of adult foods prepared by processing the ingredients to make them easily digestible. The food should be balanced and nutritious to promote healthy growth of the child.

2.2.4. Functional properties of complementary foods

In most developing countries traditional weaning foods are mainly stable cereals and starchy roots in the form of porridge or gruel which are characterized by low protein, high bulk, and low energy/unit volume food (Nkama *et al.,*1995). Guiro *et al.,*(1987) stated that one major cause of weaning-age malnutrition is complementation with cereal gruel that is of low energy density. Wondimu and Malleshi (1996) claimed that immediate cause of the prevalence of protein energy malnutrition is inadequate intake and poor density of nutrients. The concept of dietary bulk of traditional foods, as a major constraint in providing enough nutrient to small children, and as an important factor responsible for the development of malnutrition in areas where cereals and starchy stables are the main food. Physical attributes namely spread ability, bulk density, viscosity and water activity of complementary foods play an important role in determining the acceptability of the product. (Kshirsagar *et al.,*1994).

Physical characteristics *viz*., particle size, bulk density, viscosity and water activity of complementary foods and composite mixes play an important role in determining the acceptability of the product. Viscosity of the formulation was measured at 5, 10, 15 and 20 per cent hot paste slurry showed 16, 24, 56 and 92 cp respectively (Chandrashekhar *et al*., 1988). Kshirsagar *et al*., (1994) developed malted, roasted and formulated weaning mixes using finger millet, green gram, groundnut and skim milk powder at 35:35:10:20 proportion. The developed malted food (49.1 m.Pa.s) showed lowest cold paste viscosity followed by formulated + 5 per cent barley malt added food (73.6 m.Pa.s) compared to formulated (834 m.Pa.s) and roasted (1374 m.Pa.s). Suma, (1998) indicated that malted mix had lower viscosity (560 cPu) when compared to roasted (3520 cPu). The viscosity of roasted food reduced significantly (1800 cPu) with the addition of 5 per cent Amylase Rich Food.

Swamy (2003) developed complementary food with 12 parts of malted ragi, 36 parts of malted wheat, 20 parts of malted green gram and 30 parts of sugar and studied for the physical parameters bulk density and spreadability. The spray dried complementary food showed bulk density of 0.53g/ml and spreadability of 27.0 per cent compared to tray dried complementary food (0.59g/ml, 24%) and baked complementary food (0.65g/ml, 20%) respectively.

Usman*et al*., (2016) produced weaning foods from the blends of sorghum flour, malted sorghum flour and soybean flour. Inclusion of malted sorghum flour (MSF) in the weaning food blends resulted in a decrease in water binding capacity (WBC). The WBC of Samsorg 17-(M0), *Pelipeli*-(M0) and Hybrid-(M0) were 3.81, 3.51 and 3.31 ml/g. The WBC was also observed to decrease with an increase in the quantity of MSF included. The viscosity of the weaning food blends ranged from 14.32 to 33.61 cP with Hybrid-(M10) and *Pelipeli*-(M0) having the lowest and highest values, respectively. At the temperature (40°C) in which the viscosity of the weaning food blends was measured, it was observed that the viscosity of the blends generally decreased with an increase in the quantity of malt added.

2.2.5. Storage stability of complementary foods

Malted weaning formulation developed using malted finger millet, horse gram and roasted peanut at the proportion of 65:25:10 (Chandrasekhar *et al*., 1988) was evaluated for storage stability. Mix was stored in household containers like plastic containers, tins, glass bottles and polythene bags for a period of 45 days and results revealed that as storage period progressed, moisture content increased. But the per cent increase was higher in tins (6.25 to 9.02) and lower in polythene bags (6.25 to 7.39) compared to plastic containers (6.25 to 8.86) and glass bottles (6.25 to 9.02).

Four supplements were developed using cereals (wheat, pearl millet), pulses (Bengal gram, green gram) amaranth leaves and jaggery at the proportion of 4:1:1:1:4 (Dahiya and Kapoor, 1994), and were stored in three packaging materials viz., polythene bags, tins and glass bottles for a period of 30 days. As the storage period increased there was a significant increase in moisture, peroxide value, fat acidity and alcoholic acidity. Mixes stored in tins possessed higher moisture (6.85- 8.16%), peroxide value (3.65-5.3meq/kg fat), fat acidity (24.43-25.95%) and alcoholic acidity (0.083-0.124%). Pearl millet based supplements had higher moisture (7.9-8.16%), peroxide value (4.38-5.11meq/kg fat), fat acidity (25.43-25.95%) and alcoholic acidity (0.110-0.124%) on 30th day of storage. Organoleptic characteristics of all the supplements were not affected till one month of storage except the fact that pearl millet based supplements exhibited a change in taste. The packed mixes in polythene pouches, bottles and plastic containers and stored for a period of 45 days revealed that moisture content showed slight increase between zero to fifteen days of storage.

Malted weaning foods had higher peroxide values than roasted foods. However, weaning foods were judged acceptable for consumption even after 60 days storage. Storage of composite mixes results in changes in sensory and chemical parameters i.e. moisture content, free fatty acid, peroxide value and alcoholic acidity when packed in various packaging materials and stored at different environmental conditions (Gahlawat and Sehgal, 1994).

The keeping quality of the developed weaning mixes using grain amaranth and Italian millet at the proportion of 70:30 by employing roasting and malting techniques by Suma (1998) revealed that increase in moisture (4.7 to 7.6%) and peroxide value (0 to 3.5meq/kg fat) of roasted mix from 0 to 8th week of storage was less compared to malted mix (5.29 to 8.59% moisture and 0 to 2.9mEq/kg fat peroxide value) from zero to fifth week of storage.

Raza*et al*.,(2009) prepared nutritionally balanced baby foods from indigenous food materials utilizing home cooking methods and evaluate the sensory attributes and shelf life at ambient conditions for 3 months. No significant change in moisture, peroxide and free fatty acid values were observed.

Lohia and Udipi (2015) formulated complementary foods using fermented or malted locally available cereals and pulses and shelf life studies were analyzed. The moisture content and peroxide value increased during the accelerated storage. The shelf life under accelerated conditions was about six weeks from the date of manufacture. At the end of two weeks, the peroxide value exceeded the limit of 10 mEq/kg as per ISI specifications. Results of microbial analysis indicated that there was an increase in the bacterial count at Day 14 of the shelf life study, although the count was within the limit given by Food Safety and Standard Authority of India (FSSAI, 2016).

2.3. Concept, definition and historical perspective of probiotics

According to an FAO/WHO Expert Probiotics are defined as the “live microorganisms, which when administered in adequate amounts confer a health benefit on the host.”The name probiotic comes from the Greek word 'probios' which means ‘for life’. The history of probiotics began with the history of human being. Cheese and fermented milk were well known to the Greeks and Romans, especially for children and person convalescence (Lilly and Stillwell, 1965; Parker, 1974; Fioramonti, 2003; Roller 2004). Probiotics have been used in foods for millennia to transform avariety of food substratesinto fermented foods characterized by their specific sensory properties, improved stability, enhanced nutritive value, and perceived “wholesomeness” (Salminen *et al.,* 1998; Marteau *et al*., 2001; Ritzi *et al.,* 2014).

2.3.1. Health benefits of probiotics

Great interest has recently been focused on the development of cereal based probiotic foods as the global probiotic market is estimated to reach US 31.2 billion in 2014 (Anon, 2016). Probiotic bacteria and their health effects have recently become the focus for Intensive International research (Yoo and Kim, 2016). Nowadays, consumers are aware of the link among lifestyle, diet and good health, which explains the emerging demand for food products that are able to enhance human health and wellbeing beyond providing basic nutrition. The list of health benefits accredited to functional foods continues to increase day by day among which use of probiotics are one of the fastest growing categories within food for which scientific researchers have demonstrated therapeutic evidence

(Govender*et al.,* 2014; Lourens and Viljoen, 2001).Probiotic bacteria have also been widely recognized to have other health benefits to the patient such as effects on immunological functions, aiding in digestion, as well as protection against pathogenic bacteria such as *Salmonella typhimurium, Helicobacter pylori and Escherichia coli* (Penner *et al.,* 2005, Sunada *et al.,* 2008). Fig. 2.7 shows the health benefits of probiotics.

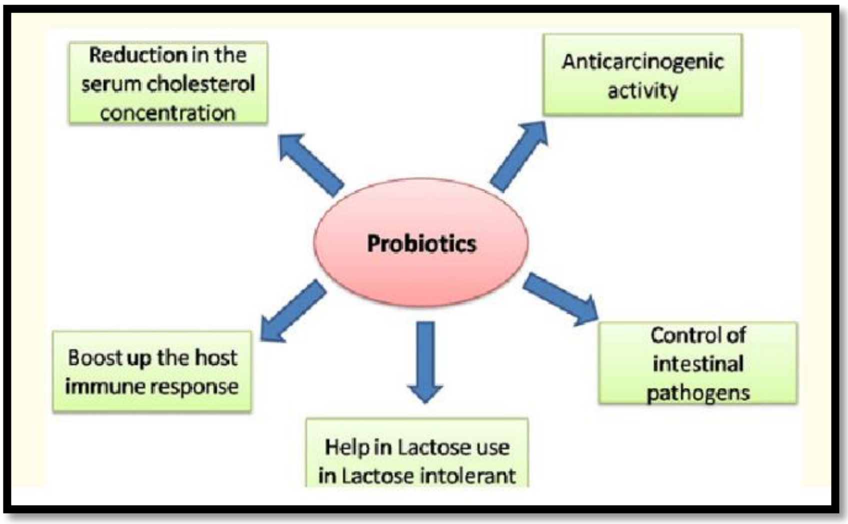


Fig. 2.7. Health benefits of probiotics
(Salminen *et al.,* 1988)

Probiotics, by definition, should adhere to the intestinal cells, not promote or encourage antibiotic resistance, not as themselves be pathogenic in nature, and must be able to co-aggregate as part of the natural gut flora (Beachey, 1981). Among all food grade probiotic stains, species of Lactobacillus and Bifidobacterium were mostly used because of their varied probiotic potentials in human gut microflora (Alander *et al.,* 2001).

The use of probiotic cultures stimulates the growth of bacteria, and reinforces the body's natural defense mechanisms. There is a growing scientific evidence to support the concept that the maintenance of healthy gut microflora may provide protection against various gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases and even cancer. The degree at which the functional benefits are received are dependent on the type of bacteria delivered as well as the number of viable bacteria that is delivered to the gastro-intestinal system (Vasiljevic and Shah, 2008).

Duffy (2000) reported that competitive exclusion (CE) is a specific type of probiotic strategy that involves the addition of a non-pathogenic bacterial 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. Probiotics constantly binds and removes pathogens from the intestinal tract by stimulating the host's immune system (Spring *et al.,* 2000). Many studies successfully revealed the effect of lactic acid bacteria in gastrointestinal micro biomeamong *in vivo* models combination of *Lactobacillus* strains possesses positive effect on Gastro Intestinal stresses in both survival and activity (Marteau *et al.,* 2001; Derrien *et al.,* 2015). In some cases, these results are also supported by validation in human subjects (Van and Veen, 2012).

Some published trials of strains like *Lactobacillus rhamnosus* was administered to mothers before delivery and then to mothers or infants until 6 months of age. It has been observed that there was a preventive effect of *Lactobacillus rhamnosus* on eczema at the age of two, four and seven years of age (Kallrorraliru *et al.,* 2003 and Keruorrin, 2007). There were no effects of *Lactobacillus rhamnosus* on respiratory allergies or sensitization, and the mechanism was suggested to be IgE-independent. In contrast, there was no preventive effect of administration of *Lactobacillus reuteri* to mothers before delivery and to infants until 12 months on eczema, but the incidence of IgE-mediated eczema was reduced (Anneneusso *et al.,* 2007)

Recent research evidenced that Lactic acid bacteria (LAB) strains are the major representatives of probiotics both in the food and pharmaceutical market which not only associated with the improvement of metabolic diseases, but were also found to scavenge hydroxyl radicals and superoxide anion, thus enhancing antioxidant activity in food as a microbial additive (Wang *et al.,* 2017). Persichetti*et al.,*(2014) stated that lactic acid probiotic bacteria possess significant free radical scavenging activity in nondairy food products by both *in vivo* and *in vitro* studies.

Roy *et al.,*(2013) also found increased free radical scavenging activity in lactic acid fermented cereal-legume based food products which is due to hydrolysis of proteins after fermentation resulted in the formation or exposure of high-affinity metal-binding groups such as, the imidazole and carboxylic groups which therefore increased the electrostatic and ionic interactions between the peptides and Cu^{2+} leading to increased antioxidant activity as those specific peptide structure and amino acid side chain groups plays an important role in terminating free radical scavenging activity.

In the last decade, a large number of EPS-generating lactic acid bacteria have been isolated from a variety of fermented food systems. However, *Lactobacillus plantarum* is found to be an eminent microorganism for its EPS-producing properties and received considerable attention (Wang *et al.,* 2010). Many studies reported that EPS produced by *Lactobacillus plantarum* has been considered as a potential grade of bioactive natural product having antioxidant effects (Hidalgo-Cantabrana *et al.,* 2012; *Zhou et al.,* 2016) probably due to presence of other antioxidant components in EPS like protein, peptides and microelements, These compounds exhibit potent antioxidant efficacy by interacting with similar components present in fermentation medium (Seo *et al.*, 2015). LAB derived EPS can protect the microbial cells against desiccation, phagocytosis, phase attack, antibiotics or toxic compounds (Liu *et al.,* 2011). Polak *et al*.,(2013) reported that the antioxidant effects of EPS are assumed to be due to its structure-function and composition relationship in EPS biopolymers which play a crucial role for their specific biological actions.

2.3.2. Probiotic strains

The use of probotic strains in food formulations can be dated back decades with the first documented article of bacterial supplementation published in the early twentieth century by Elie Metchnikoff. Probiotic research has, however, greatly increased in the last decade with over 5,000 publications detailing their health benefits as well as their ability to delivery viable functional probiotic bacteria (Verna and Lucak, 2010). In the food industry, probiotics have been widely used in the dairy sector, such as yogurt-like products. The quick rise in this market sector may be associated with a better understanding and acceptance by the consumer of the presence of viable microorganisms within these traditional fermented products (Annunziata and Vecchio, 2011). Table 2.1 shows the microorganisms that are considered as probiotics by Holzapfel, (2013)

Table 2.1. Microorganisms considered as probiotics

Microorganisms considered as probiotics	
Lactobacillus species	**Bifidobacterium species**
L. acidophilus	*B. adolescentis*
L. casei	*B. animalis*
L. crispatus	*B. bifidum*
L. gallinarum	*B. breve*
L. gasseri	*B. infantis*
L. johnsonii	*B. lactis*
L. plantarum	*B. longum*
L. paracasei	
L. reuteri	
L. rhamnosus	

The last years have also witnessed an expansion to non-dairy probiotic products, as for example sausages, fruit juices, chocolates and cereals; however, the success of such products is still small either due to poor market share or to technological hurdles. Nonetheless, it is commonly accepted that the incorporation of probiotics into a food product increases its commercial value, as shown by recent market studies (Euromonitor, 2010) which describe an increasing awareness and preference of the general consumer for functional foods that incorporate probiotics.The main species of bacteria used in probiotic formulations are *Lactobacillus* and *Bifidobacterium* spp., which are classified as anaerobic bacteria and therefore require an oxygen-free environment for growth to occur (Cook *et al.,* 2012; Maragkoudakis *et al.,* 2006).The probiotic potential of different bacterial strains, even within the same species, differs from each other. Different strains of the same species are always unique, and may have differing areas of adherence (site-specific), specific immunological effects, and actions on a healthy vs. an inflamed mucosal milieu may be distinct from each other.

Current probiotic research aims at the characterization of the normal, healthy gut microbiota in each individual, assessing the species composition as well as the concentrations of different bacteria in each part of the intestine. According to Shah (2007) the most popular strains used in food industry are represented by the following genera: *Lactobacillus, Streptococcus* and*Bifidobacterium,* but there are some other organisms like *Enterococcus* spp.

and *Saccharomyces* spp. which have also been used as probiotics. Other probiotic strains

***Enterococcus* spp.**

Enterococci belong to the lactic acid bacteria (LAB) and they are of importance to foods due to their involvement in food spoilage and fermentation. They are gram-positive, catalase negative and have the ability to convert glucose into lactic acid as the main product of primary metabolism. They donot produce spores, are oxidase negative and facultative anaerobes. They are regular commensals of the gastrointestinal tract, the oral cavity and the vagina in humans (Fritzenwanker *et al*., 2013). Currently, 37 species of *Enterococcus* are validly described (Holzapfel, 2013), which fall into seven species grouped on the basis of 16 rRNA gene similarity. These microorganisms are used as starter cultures in food products, such as cheese, as probiotic cultures for humans and animals and as silage additives (Gaggia *et al*., 2010).

2.3.3. Probiotic strains inoculated complementary food mixes

Baldeon*et al.,* (2008) and Suharja *et al*., (2012) reported that formula with probiotics was well tolerated and safe for all participating children. *B. Lactis BL* and *S. Thermophilus* present in the formula with probiotic were viable and susceptible to culture. There was difference on physical growth or development among children who were supplemented with formula with probiotic, or regular family food. Inadequate growth could be the result of the frequent acute intestinal and respiratory infections observed in all participants.

Semwal *et al*., in 2015 developed weaning mix from underutilized crops of Uttarakhand (finger millet, barnyard millet, black soybean, amaranth grain). Malting and fermentation were adopted as ways of improving cereal protein quality and decreasing anti-nutritional property and probiotic weaning mix was produced using probiotic strain *Lactobacillus plantarum*. The probiotic fermentation resulted in favorable changes in nutritional profile of weaning mix resulting 48.2% increase in protein and 92.3% decrease in tannin content.

2.4. Research studies on Probiotification

2.4.1. Evaluation of antidiarrhoeal activity by supplementing probiotics through animal studies

In-vivo studies using animal models have contributed to a great understanding in the field of medical and nutrition research (Rosac, 2016). Animal models are frequently employed in the study of human diseases, because of their similarity to human being in terms of genetics, anatomy, and physiology.

Livingstone *et al*.,(1993) developed malted and popped complementary food with wheat, chickpea, skim milk powder and sucrose in the ratio (60:30:5:5) and evaluated for its nutritional value. The malted food showed higher values for Net Protein Ratio (NPR), Protein Efficiency Ratio (PER), Biological Value (BV) and Net Protein Utilization (NPU) which was 4.00, 2.91, 88.3 and 77.3 compared to the popped food NPR, PER, BV and NPU which was 3.94, 2.68, 86.7 and 71.9. Complementary food was developed using wheat and soybean in the ratio of 70:30 and evaluated for protein quality. The mix showed PER of 2.21, BV of 90.17 and NPU of 82.20 (Begum and Kupputhai, 1993).

Srivatsava *et al*. (2015) developed the malted and popped proso millet based complementary food with 70 parts proso millet, 15 parts roasted peanut flour and 15 parts roasted soy flour and assessed it for protein quality evaluation taking casein diet as control. The malted mix reported higher PER of 2.13 compared to popped 1.88 while that of casein diet was 2.50.

The complementary food developed using 12 parts of malted ragi, 36 parts of malted wheat, 20 parts of malted green gram and 30 parts of sugar with and without whey protein concentrates (WPC) added at 20 per cent was evaluated for its nutritional quality by assessing the PER, FER and NPR taking skimmed milk powder as control. The malted complementary food with WPC showed higher values of PER, FER and NPR (3.20, 0.31, 3.06) compared to malted food without WPC (2.50, 0.24, 2.03) and control (2.98, 0.30, 2.94) respectively (Swamy, 2003).

A rat bioassay was conducted to pre-clinically evaluate the nutritional quality of 2 supplementary foods (SF) based on corn and soy blends for feeding preschool children. Groups of male weanling rats were fed with SF for 4 weeks to evaluate the protein quality. Results showed that the body weight gain of rats fed with SF were significantly higher than those fed with skim milk powder diet (control). The PER and NPU results of SF were not significantly different ($p>0.05$) from values of the control group it was concluded that these SF are nutritionally comparable to skim milk powder (Baskaran *et al*., 2001).

Weaning food formulated from germinated corn flour, legume meal and sesame seed meal compared with non-germinated blends as weaning foods for infants. Weanling rats weighing 55-65 g were given feeds containing germinated or non-germinated flour for a period of 28 days. The germinated flour diets appeared to be more palatable to the rats, leading to higher feed intakes. (Ikujenloa and Fashakin, 2005).

The nutritional quality of the wheat based complementary food with malted wheat flour, skimmed milk powder, groundnut oil and sugar was assessed by an *in-vivo* study on experimental male weanling rats for a period of 28 days. The complementary food with 13.8 per cent protein, 7.55 per cent fat, 72 per cent carbohydrate and 1.78 per cent ash showed a feed efficiency ratio of 0.29 (Jayashri *et al*., 2012; Chunmei *et al.,* 2010)

Accordingly, this study displayed a dose dependent reduction in percentages of total fecal output ($p<0.001$) indicating the antidiarrhoeal potential of the study models. This study is concordant with other studies 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 (Chandra,2002).

Another double-blind randomized study investigated that Lactobacillus reuteri DSM 17938 significantly reduced watery diarrhoea than placebo (2.1±1.7 days vs. 3.3±2.1 days; $P<0.03$) as well as relapse rate of diarrhoea (15% vs. 42%; $P<0.03$). Foster *et al.,*(1980) reported that, injection of Lactobacillus preparation in infected ileal loop significantly reduced the enterotoxin response against Escherichia coli entero toxin-induced diarrhoea in the rabbit (Francavilla *et al.,* 2012).

Another study reported that 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 which explain the similarity of the findings of this present study (Foster *et al.,* 1980).

2.5. Validity of yardsticks used in present investigation

Several methods were used in this study depending upon the objectives of experiment. These methods used were found to efficient and are supported by the reviewed references:

2.5.1. Freeze drying

The process of freeze drying was invented in 1906 by Jacques Arsened Arsonval and his assistant Frederic Bordas at the laboratory of biophysics of College of France in Paris. Freeze drying is technically known as Lyophilization or Cryo-desiccation. It is a dehydration process typically used to preserve a perishable material or make the material more convenient for transportation. It is the removal of ice or other frozen solvents from a material through the process of sublimation and the removal of bound water molecules through the process of desorption.

Principle of Freeze drying

Freeze drying is based on the principle Sublimation i.e. When a solid (ice) changes directly to a vapour without first going through a liquid (water) phase. Freeze drying has been a method of choice for the long- term preservation of bioactive materials. This dehydration method causes little shrinkage and results in a completely soluble product that is easily rehydrated. Moreover, lyophilization is frequently used to preserve lactic acid bacterial starter cultures involved in dairy and food fermentations (Lodato *et al.,* 1999). But, not all strains survive the process (Abadias *et al.,* 2001). The major causes of loss cell viability in freeze-drying are probably ice crystal formation and high osmolarity (Conrad *et al.,* 2000). Microbial cell survival during the freeze drying process is dependent on many factors, including protective additives and conditions during rehydration (Font de Valdez *et al.,* 1983). Many compounds have been tested to improve the survival of lactic acid bacteria during freeze drying, including polysaccharides, disaccharides, amino acids and proteins (Champagne *et al.,* 1991).

These compounds were in most cases found to be effective toward protection of different lactic acid bacteria (Leslie *et al.,* 1995; Linders *et al.,* 1997; Carvalho *et al.,* 2002; Zayed and Roos, 2004)

Freeze drying and nutrient retention

From a commercial point of view, an inexpensive method for large-scale production of cultures containing high levels of viable probiotic cells in a form suitable for product applications is highly desirable. Dehydration is a commonly used preservation method to stabilize probiotics for their ease of storage, handling, transport and subsequent use in food and pharma industry. Freeze drying and spray drying are the most common downstream processes used for the preparation of dried stable probiotic cultures (Madhu and Awasthi, 2011). The importance of starters in dairy industry is apparent from the industrial point of view (Prasad *et al.,* 2003). Freeze-drying is the most widespread technique for dehydration of probiotic and dairy cultures, while spray drying has been applied to the dehydration of a limited number of probiotic cultures (Meng *et al.,* 2008).

As processing conditions addition, associated with freeze drying are milder than spray drying, higher probiotic survival rates are typically achieved in freeze dried powders (Wang *et al.,* 2004).Chavez and Ledeboer (2007) found freeze drying as the most appropriate process for survival of probiotic bacteria even after long storage.

2.5.2. Sensory evaluation

Sensory evaluation has been defined as a scientific discipline used to evoke, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Sidel and Stone, 1993).

Gupta *et al.,*(1992) formulated weaning mixtures from malted and roasted pearl millet, barley, amaranth and green gram. Two mixes formulations were prepared by mixing pearl millet or barley with amaranth, green gram and jiggery in the ratio of 60:20:40:45. Both the formulations were rated ‘like moderately’ by a panel of ten judges with a score of 6.66 and 6.88.

Complementary mixes were prepared by Premakumari *et al.,* (2011) comprising of cereal, grain amaranth and dehydrated leafy vegetable in a ratio of 7:2:1. In the mixes containing puffed or flaked rice, part of amaranth and leafy vegetable were replaced by skim milk powder in the ratio of 14:3:2:1 (cereal: amaranth: skim milk powder: leafy vegetable). The mixes when evaluated for acceptability, results indicated that four mixes were most acceptable (malted wheat +malted amaranth +spinach; raw rice+ roasted amaranth+ spinach; puffed rice+ puffed amaranth+ skim milk powder+ spinach; rice flakes+ puffed amaranth+ spinach).

Six mixes were formulated using pre-gelatinised maize, sweet potato, soybean and peanuts (Idowu *et al*., 1993). Soybean and defatted peanut flours were used at 25 and 5 per cent respectively and evaluated for sensory quality in the form of porridge. The blend of 20:50 of maize and sweet potato was most acceptable, among all the mixes. Flour blends of rice and green gram were prepared at the ratio of 100:0, 80:20, 60:40, 50:50, 40:6, 20:80 and 0:100. To each of the blend sesame and carrot flours of 5g each were added. The blends were subjected to sensory evaluation in the form of gruels and results revealed that among the blends 50:50 was most acceptable with the overall acceptability scores of 6.8 (Pawar and Dhanvijay, 2007).