

## Development of Symbiotic Yoghurt

Shireesha B, Panchala Raju M, Shobha S and Aparna Kuna

Department of Foods & Nutrition, Post Graduate & Research Centre, Acharya N.G.Ranga Agriculture University, Hyderabad-500030, India

---

**Abstract:** The product was developed with both probiotic and Prebiotic incorporation. The probiotics used were *Lactobacillus Bulgaricus* and *Streptococcus Thermophilus* as live starter cultures and the prebiotic was Fructo – oligosaccharide. In addition to this Sweet potato was also added for the stabilization of yoghurt. So the study was conducted to develop a symbiotic yoghurt incorporating sweet potato, to know the consumer acceptability, to determine the shelf life, to evaluate the physico chemical and nutritional parameters of product. In the research work product was formulated and standardised with live probiotics and prebiotic combination. The product formulations were done in three combinations with different proportions of ingredients. As T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, the proportions prepared as T<sub>1</sub> ( Plain yoghurt ), T<sub>2</sub> ( Plain Yoghurt + FOS ), T<sub>3</sub> ( Live Active Culture 3%). So the double toned milk was used for the development of low fat yoghurt. The milk was pasteurized, homogenised and stabilized with boiled and mashed sweet potato divided into three samples with different formulations. The respective formulations are made and kept in incubator for 3 hours, at 41 – 42<sup>o</sup>C and immediately transferred to refrigerator, stored at 4<sup>o</sup>C for further analysis. All the three formulations were subjected to evaluation for Physico chemical and nutritional analysis. Microbial analysis was done to know the shelf life of product. The microbial count estimation were done for bacteria, yeast and Mould count for twice keeping the product for 21 days of storage life. The results from sensory analysis showed that there was a difference in colour attribute T<sub>1</sub> sample was more attractive than T<sub>2</sub> and T<sub>3</sub>. In flavor T<sub>3</sub> scored high compared to T<sub>1</sub> and T<sub>2</sub> as the live active culture incorporated in 1:1 ratio. In appearance T<sub>2</sub> scored more than T<sub>1</sub> and T<sub>3</sub>. In texture and acceptability T<sub>3</sub> sample was the best accepted from consumers. The product T<sub>3</sub> sample yoghurt was the best suited with correct proportion of ingredients, 30% sweet potato was suited for good quality product development.

**Keywords:** Probiotic and Prebiotic, Symbiotic yoghurt.

---

### I. Introduction

Consumer interest in healthy eating is shifting towards the potential health benefits of specific foods and food ingredients. Moreover, scientific evidence supports the idea that some of these might have positive effects on our health and well-being, beyond the provision of basic nutritional requirements. Over the last few decades the process has become more rational, mainly due to various discoveries and/or improvements in such disciplines as microbiology and enzymology, physics and engineering, chemistry and biochemistry. Besides this the population concerned the elderly are increasing in number. Various age related changes are seen in this group ageing related changes to the digestive system can lead to gastrointestinal disorders or simply digestive discomfort (Donini et al., 2009) such as bloating, flatulence, abdominal pain and altered bowel habits (Guyonnet et al., 2007), hypochlorhydria because of atrophic gastritis, leads to a decreased absorption of calcium, ferric iron and vitamin B12 (Russell, 1992), reducing micronutrient intake. Furthermore, decreased intestinal motility resulting in faecal impaction and constipation is a major problem in elderly people. The current trend of new promising technologies such as nutrigenomics, imaging techniques, converging technologies are increasingly being used in nutrition research to reduce the risk of health problems for individuals. So to design and to standardize, a food suitable and beneficial to a particular age group is an essential criteria for the food technologists. Dairy is gaining in popularity, driven in large part by innovations in yoghurts. By now, enough consumers are likely to be aware of the helpful bacteria naturally present in yoghurts, increasing receptiveness to the idea of probiotic and prebiotic yogurts.

Inulin, oligofructose, lactulose, galactooligosaccharides and synthetic fructo-oligosaccharides (FOS) are probably the only prebiotics for which available scientific evidence indicate limited and defined health benefits. The chemical structure of these prebiotics prevents their digestion in the small gut. Consequently, they reach the large bowel undigested and are fermented by bacteria.

Yoghurt is defined as —product obtained by the fermentation of milk with cultures of streptococcus thermophilus and lactobacillus delbrueckii ssp. bulgaricus, however, —yoghurt-like products are made by substituting *Lactobacillus bulgaricus* by other *Lactobacillus* species for the fermentation of milk or yoghurt containing probiotic bacteria.

Ageing related changes to the digestive system can lead to gastrointestinal disorders or simply digestive discomfort (Donini et al, 2009) such as bloating, flatulence, abdominal pain and altered bowel habits (Guyonnet et al, 2007). Age related changes in the gastrointestinal tract combined with changes in diet and immune system reactivity affect the composition of gut microbiota, leading to increased numbers of facultative anaerobes, decreased number of beneficial organisms like anaerobic lactobacilli and bifidobacteria and the consequence of these changes can be an impaired digestive function with increased transit time, increased putrefaction of the colon and a greater susceptibility to disease (Donini et al, 2009). “A normal type of food with an additional ingredient that provides a health benefit beyond satisfying traditional nutritional requirements” (Food-info.net, 2010) Increasing dietary fibre along with the use of probiotic or prebiotic supplements or functional foods, have been suggested to improve digestive and immune health in older people (Donini et al, 2009).

A Probiotic is defined as “A live microbial supplement, which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989).

Schiffirin et al (2009) report on a study of probiotic yoghurt containing a *Lactobacillus johnsonii* strain on older independently living people (median age 71) that showed normalisation of the response to endotoxin and modulation of activation markers in blood phagocytes, which may help to reduce low grade chronic inflammation.

#### **Potential Benefits of Probiotics:**

- Restore the normal balance of microbes in the intestines
- Treatment of diarrhea
- Treatment of gastroenteritis
- Alleviate some of the symptoms of irritable bowel syndrome such as: Constipation, Diarrhea, Abdominal pain, Flatulence, Bloating, Improve immune function.
- Improve lactose digestion and tolerance

Vouloumanou et al. (2009) evaluated the clinical evidence regarding use of probiotics for prevention of respiratory tract infections and concluded that probiotics appear to have a beneficial effect on the severity and duration of symptoms of respiratory tract infections but do not appear to reduce the incidence of such infections. Probiotic treatments have also been used to effectively treat antibiotic associated diarrhea (Agrawal, 2005).

#### **Potential Benefits of Prebiotics**

- Improve bowel function
- Increase stool frequency
- Increase stool weight
- Increase production of short-chain fatty acids
- Promote the growth of the health promoting bacteria Lactobacilli and Bifidobacteria
- Restore gut flora during or after antibiotic Therapy
- Inulin can reduce insulin concentrations and lowered triglyceride levels

Stephen Olmstead et al (2008) stated that Prebiotic augmentation of both gastrointestinal and systemic immunity can enhance pathogen resistance in the intestinal tract and may reduce the risk of infections throughout the body. Symbiotics are defined as mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host (Andersson et al., 2001). Fructooligosaccharides (FOS) is a soluble dietary fiber naturally found in a variety of fruits, vegetables, and grains such as bananas, barley, garlic, honey, onion, rye, brown sugar, tomato and asparagus root. It acts as a food source for friendly intestinal bacteria and enhances calcium absorption. The most important prebiotics are glucans, fructans and mannans. Among the fructans, inulin and oligofructoses are commonly used (Oliveira et al., 2009a). Fructo-oligosaccharide, or FOS, is only a type of prebiotic. The word fructo- oligosaccharide literally means small (oligo) sugar (saccharide) that contains fructose (fructo). Recommended daily intake of FOS for humans is 4g (Roberfroid et al.1998). The prebiotic FOS does not have to be refrigerated and has a 3-year shelf life. Lewis et al. (2005) found that FOS demonstrated antagonistic effects in humans with ulcerative colitis. *Lactobacillus bulgaricus* probiotic activity can be ascribed to its ability to produce substances with antimicrobial properties and Lactobacilli are known to inhibit the growth of pathogenic bacteria, possibly by producing inhibitory compounds such as organic acids, hydrogen peroxide and bacteriocins (Loessner et al., 2003). *Streptococcus thermophilus* is physiologically and biochemically less versatile than other lactic acid bacteria, the reality is that this organism is actually very versatile.

The present study was undertaken to standardise a product called “Symbiotic Yoghurt” with natural probiotics and prebiotics as a symbiotic combination.

## II. Materials And Methods:

The present study was carried out to develop a symbiotic yoghurt (Combination of Probiotics and Prebiotics) with the incorporation of sweet potato and was done in three stages which includes,

- I. Propagation of freeze dried culture into live active starter culture.
- II. Standardization of product.
- III. Evaluation of the product.

### Location of Study:

The various materials and methods required for the study on “Development of Symbiotic Yoghurt for elderly people” are discussed below. The study was conducted in the Department of Food Technology, Post graduate and Research center and Quality Control lab, Acharya N.G Ranga Agricultural University, Rajendranagar, Hyderabad.

### Materials and chemicals:

For the present study, doubled toned milk and skim milk powder were purchased from Heritage Foods India Ltd (Hyderabad, India), and sweet potato was purchased from local market (Hyderabad, India). Fructo-oligosaccharide obtained from Morde Foods Private Ltd (Mumbai, India). All other chemicals were purchased from Qualigens Fine Chemicals (Mumbai, India) or Molychem India Pvt. Ltd. (Mumbai, India). Microbiology media were obtained from Hi-Media Laboratories (Mumbai, India). The cultures required for product development were purchased from National Chemical Laboratory, Pune. Unless otherwise mentioned all chemicals used were of analytical grade.

### Physico-chemical and nutritional characteristics:

Total fat, SNF (Solid Not Fat), Specific gravity, Total solids and pH of developed symbiotic yoghurt samples were determined as per the AOAC methods (AOAC, 2002). Acidity was calculated by titrating against 0.1 N NaOH and expressed as percentage of lactic acid. Protein, Carbohydrates, total sugars, calcium and phosphorus and dietary fibre were determined using approved AOAC methods (AOAC, 2002).

### Propagation of pure culture:

From the freeze dried pure culture microbes were isolated by inoculating in suitable media, for *Lactobacillus bulgaricus* in de Man's Rogosa and Sharpe (MRS) broth and for *Streptococcus thermophilus* in Nutrient broth with proper sterilization by keeping in autoclave at 15 psi, 121°C, for 15 minutes, to kill all the vegetative cells and spores in glassware. The sterilized liquid media was filled in broth tubes and incubated for 24 hrs at 37 °C. The growth developed in broth tubes was enumerated for viable count by pour plate method with the semi solid media by adding agar to it. The growth observed in broth is subjected to centrifugation at 4500 rpm for 15 minutes, collected the pellet and discarded the supernatant, done the serial dilution and added 1ml from broth to semisolid media in petri plate by pour plate method in laminar air flow chamber to avoid contamination. The plates were kept in incubator for 24 hrs at 37 °C, the microbial count was observed in 10<sup>-6</sup> dilution which was the required CFU count for yoghurt preparation. Colonies found on MRS agar plates were white in color, convex in shape, small in size, pinpoint, smooth and 1- 2.2 mm in diameter; whereas, there was turbidity, sedimentation and small white suspensions in MRS broth.

### Standardisation of product:

The yoghurt was manufactured according to international standards of yoghurt manufacture (IDF, 1983 standards). The milk was homogenized with sweet potato which was boiled and mashed and heated to 85°C for 3 min for pasteurization, then cooled to 43- 44°C. It was then inoculated with 3% of a mixed lactic starter (*Streptococcus salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus*). Yoghurt samples were elaborated of quantities of 100 ml for each sample and the experiment was carried out in triplicate. The inoculated milk is incubated at 41 - 42°C until a pH of 4.6 was attained in approximately 3 - 4 h (the pH end point). When the pH end point was achieved, the yoghurts were cooled at 6°C and stored at the same temperature during all period of post-acidification (for 21 days) for further analysis.

### Addition of Skim milk powder:

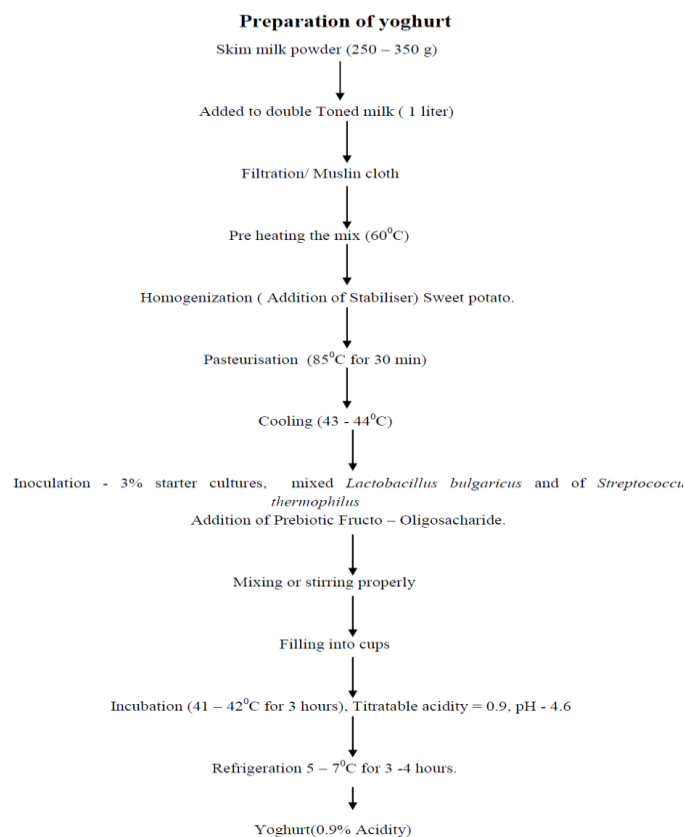
Milk powder (full cream or skimmed) is widely used in the industry to fortify liquid milk for the manufacture of thick smooth yoghurt (Bøjgaard, 1987). Since the majority of the commercial yoghurt produced is of the low fat type, it is probable that skimmed milk powder (SMP) is the more popular ingredient. The rate of addition to the yoghurt mix may range from as little as 1% to as high as 6%, but the recommended level is 3-4%, since the addition of higher levels of milk powder may lead to a powdery taste in the yoghurt.

### Addition of Stabilisers:

The primary aim of adding stabilisers to the milk base is to enhance and maintain the desirable characteristics in yoghurt, for example, body and texture, viscosity or consistency, appearance and mouth feel. The stabiliser in the present study used was sweet potato, which was boiled and thoroughly mashed. Stabilisers are sometimes referred to as hydrocolloids and their mode of action in yoghurt includes two basic functions: first, the binding of water and second, promotion of an increase in viscosity.

### Homogenisation:

According to the review by Tamime and Deeth (1980), the homogenised fat globules act as large casein micelles (i.e. because the membrane consists mainly of caseins) which increase the effective casein concentration, and hence, participate in casein reactions such as acid precipitation, the increased number of small fat globules enhances the ability of the milk to reflect light and, as a result, the fermented milk appears whiter and the risk of syneresis (i.e. separation of free whey onto the surface of set fermented milk) is reduced, and the firmness of the end product is increased giving it a better mouth feel.



### Heat treatment:

Heating of milk is an important processing variable for the preparation of yogurt since it greatly influences the physical properties and microstructure of yogurt. In yogurt manufacture, milk was heated prior to culture addition. The temperature/time combinations for the batch heat treatments that are commonly used in the yogurt industry include 85°C for 30 min or 90-95°C for 5 min (Tamime and Robinson, 1999).

### Inoculation of Cultures:

Starter cultures were inoculated as the temperature of milk reaches to 43 – 44 °C Inoculating the milk with a bacterial culture of 3% in which *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are the important organisms. The reason(s) for selecting the combinations of starter cultures used during the manufacture of yoghurt and related fermented milk products are to achieve the desired flavour characteristics of the product, mainly lactate, aroma compounds (acetaldehyde, acetone and diacetyl) and EPS (Exopolysaccharide) and to provide the consumer with a wide choice of therapeutic products.

### Fermentation process:

After heat treatment, the milk base was cooled to the incubation temperature used for growth of the starter culture. An optimum temperature of the thermophilic lactic acid bacteria, i.e., *Streptococcus* subsp.

thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus*, is around 41- 42°C for 3 – 4 hrs. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk. During acidification of milk, the pH decreases from 6.7 to ≤4.6. During fermentation, these strains synthesize different polysaccharides which contribute to the high viscosity of yoghurt.

#### Cooling:

When yogurts have reached the desired pH (e.g., ~4.6), yogurts are partially cooled. Yoghurt products are often blast chilled to <10°C (e.g., 5°C) in the refrigerated cold store to reduce further acid development (Tamime and Robinson, 1999).

#### Sensory analysis:

The sensory assessments were conducted at the Post Graduate & Research Centre, Acharya N.G. Ranga Agricultural University, Hyderabad. A panel of 12 members consisting of staff and students of university evaluated the products. To ensure that there was no bias towards the products, it was ensured that the panelists chosen were naive to project objectives. Prior to sensory evaluation the yoghurt samples were chilled to 10°C. Samples were coded using random three-digit numbers and served chilled. 25 mg of each sample was served, with the order of presentation counter balanced. Panelists were provided with a glass of water and, instructed to rinse their palate with water and drink water between samples. They were given written instructions and asked to rate the coded samples on color, flavor, appearance, texture and overall acceptability, using a nine-point hedonic scale [1=like extremely to 9 =dislike extremely] (Carr et al.1999).

#### Microbiological analysis of yoghurt:

Yoghurt which was developed by fermentation of milk with two bacteria, *L. bulgaricus* and *S. thermophilus*, which act together. For the product shelf life study was done by microbial analysis of formulated three samples. Analysis of microbial count was done by estimating: Total Bacterial count (TBC), Total Mould count (TMC) and Total Yeast Count (TYC). For enumeration of bacterial count and yeast, mould count the chemicals used were Potato Dextrose agar and HIVE Malt dextrose Agar respectively. The chemicals were prepared into media with addition of agar 2g/100 ml, sterilized keeping in autoclave and poured into petri plates by pour plate technique and kept in incubator for microbial analysis. For enumeration of bacterial count incubation period is of 5 days at 25 °C and for mould and yeast count incubation period was 24 hrs kept at 37 °C. Microbial analysis was done twice one on first day of developing yoghurt and second after 21 days of storage period

### III. Results And Discussion

Yoghurt is a gel of viscous appearance, formed as a result of the microbial acidification of milk. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria, takes part in its acid lactic fermentation and have to be in a 1:1 ratio for an effective symbiotic action. So the present study was undertaken to obtain the best and most nutritive product with the incorporation of sweet potato in the product.

#### Different formulations of product:

Table 1. Formulations used in the three products T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>.

Ingredients	T <sub>1</sub> (Control)	T <sub>2</sub>	T <sub>3</sub>
Milk	100ml	100ml	100ml
FOS	-	1.5g	1.5g
SMP	16g	16g	16g
Sweet potato	10g	10g	10g
Plain Yoghurt	5g	5g	-
Live Culture	-	-	3%

The above formulations are done from a quantity of 900 ml milk (600 ml milk + skim milk powder). Each sample labeled as T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> contained 300ml of milk taken in duplicates. Sample T<sub>1</sub> was incorporated with Plain Yoghurt, T<sub>2</sub> was incorporated with a combination of plain yoghurt and FOS, T<sub>3</sub> was incorporated with of live active starter culture. After respective formulations were made the products were kept in an incubator at 42 °C for 3 – 4 hrs until the pH reaches to 4.6 and then stored at 4 – 6 °C in refrigerator for further analysis.

**Physico chemical and Nutritional Analysis:**

Parameters	T <sub>1</sub> (Control)	T <sub>2</sub>	T <sub>3</sub>
Total solids (%)	15.5%	15.1%	13.55%
SNF (%)	12.4%	12.6%	12.05%
Specific gravity	1.044	1.047	1.044
Titratable Acidity	0.89	0.92	0.9
Protein (g)	7.15g	7.14g	12.8g
Carbohydrates (g)	12.05g	12.03g	13.9g
Fat (%)	2.5%	2.48%	1.5%
Calcium (mg)	291.07 mg	287.77 mg	289.51 mg
Phosphorous (mg)	230.15 mg	227.55 mg	229.4 mg
Dietary fibre (g)	0.061	0.0603	0.1143
pH	4.5	4.3	4.6

In the above table, the parameters for three samples as T<sub>1</sub> (Control), T<sub>2</sub> (Plain yoghurt with FOS), T<sub>3</sub> (Live starter culture) were analyzed. Total solids percentage was less in T<sub>3</sub> when compared to T<sub>1</sub> and T<sub>2</sub> because the product is developed with Double toned milk and skim milk powder combination. According to Tamime and Robinson (1999) in Yoghurt science and Technology, autoclaved, reconstituted skimmed milk (10–12g total solids (TS) 100g<sup>-1</sup>) is mainly used for yoghurt preparation and the milk must be free from any inhibitory substances, for instance antibiotics. Solids non fats are also less in T<sub>3</sub> sample when compared to T<sub>1</sub> and T<sub>2</sub>. The elevated Solids Not Fat (SNF) content of low fat yogurts forms strong casein bonds uncharacteristic in a full fat yogurt, where homogenized fat globules are partly covered with casein, facilitating protein-protein interactions. Specific gravity was also less when compared to T<sub>1</sub> and T<sub>2</sub>. Titratable Acidity which is the percentage of lactic acid fermentation was also less due to incorporation of natural live starter culture if the value is more it leads to more and rapid acidification which changes the taste of yoghurt. In the present study it was observed that TA values of 0.81 – 1.19% lactic acid which is near to the acceptable range set by the International Dairy guidelines were observed in all three samples. Protein and CHO content was 12.08 g and 13.9 g in T<sub>3</sub> comparatively T<sub>1</sub> and T<sub>2</sub> had lower levels of these nutrients. Fat percentage of T<sub>3</sub> was 1.5% which is less when compared to T<sub>1</sub> and T<sub>2</sub>. For T<sub>3</sub> sample protein present in yogurt tends to be more readily digested than the proteins present in milk. This is due to the pre-digestion of milk proteins that occurs through the action of the bacteria present in yogurt. The milk proteins in yogurt also have a higher content of the amino acids proline and glycine compared with milk and these proteins have additional functions in the body including enhancing calcium absorption and boosting the immune system. It is also important to note that the nutritional value of milk proteins is not affected by the fermentation process. The lactose in milk provides the energy source for the yoghurt starter organisms, but the protein plays an important role in the formation of the coagulum and hence the consistency/viscosity of the product is directly proportional to the level of protein present (Tamime and Robinson 1999). Calcium and phosphorous content of all the three samples were almost same. The dietary fibre was more in T<sub>3</sub> when compared to T<sub>1</sub> and T<sub>2</sub> this might be due to the stabilization with sweet potato. pH was maintained at 4.6 until the end of storage period of 21 days for T<sub>3</sub> when compared to T<sub>1</sub> and T<sub>2</sub>.

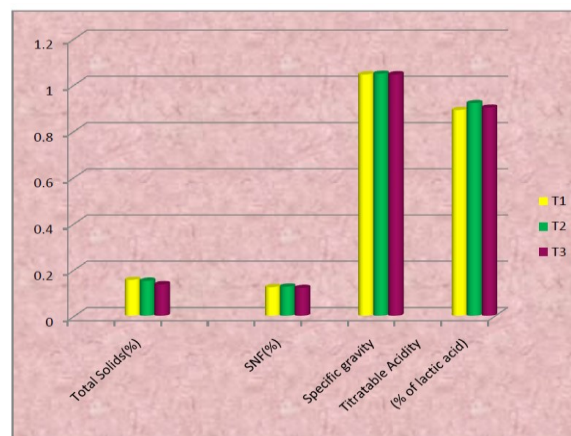


Fig.1 Graphical representation of dairy quality parameters

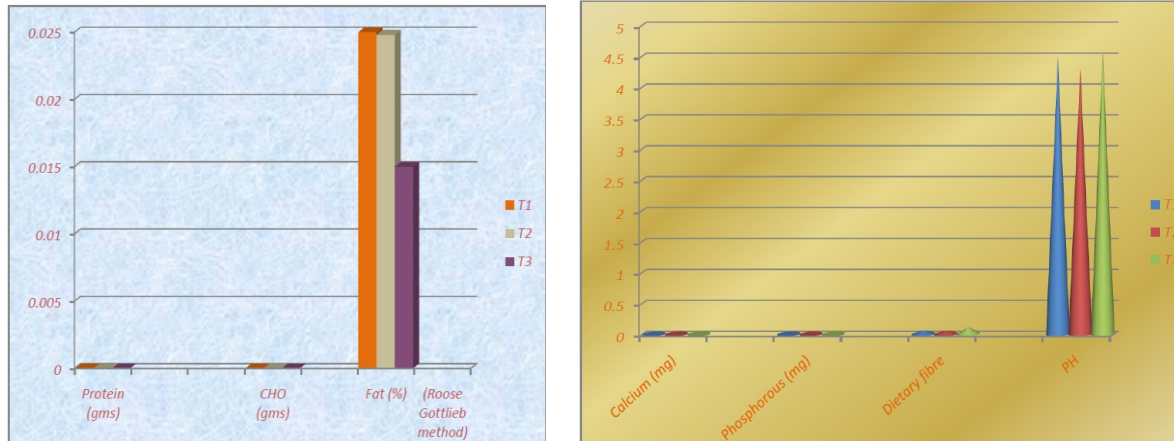


Fig.2 Graphical representation of nutritional parameters.

**Shelf Life Study:**

In the shelf life study of samples T1, T2 and T3 the microbial analysis was done with respective reagents. For the estimation of total mould count, total yeast count and total bacterial count, malt extract agar and potato dextrose agar were used respectively.

**Microbial Analysis of yoghurt on day of preparation:**

Table 3: Microbial Analysis of samples on 1st day of product preparation

Sample & Dilution	Yeast (CFU/ml)	Mould (CFU/ml)	Bacteria (CFU/ml)
<b>T<sub>1</sub></b>			
10 <sup>-1</sup>	Nil	13	Nil
10 <sup>-2</sup>	Nil	Nil	Nil
10 <sup>-3</sup>	Nil	Nil	Nil
10 <sup>-4</sup>	Nil	Nil	Nil
10 <sup>-5</sup>	Nil	Nil	Nil

Sample & Dilution	Yeast (CFU/ml)	Mould (CFU/ml)	Bacteria (CFU/ml)
<b>T<sub>2</sub></b>			
10 <sup>-1</sup>	Nil	12	Nil
10 <sup>-2</sup>	Nil	Nil	Nil
10 <sup>-3</sup>	Nil	Nil	Nil
10 <sup>-4</sup>	Nil	Nil	Nil
10 <sup>-5</sup>	Nil	Nil	Nil

Sample & Dilution	Yeast (CFU/ml)	Mould (CFU/ml)	Bacteria (CFU/ml)
<b>T<sub>3</sub></b>			
10 <sup>-1</sup>	Nil	Nil	Nil
10 <sup>-2</sup>	Nil	Nil	Nil
10 <sup>-3</sup>	Nil	Nil	Nil
10 <sup>-4</sup>	Nil	Nil	Nil
10 <sup>-5</sup>	Nil	Nil	Nil

**Microbial analysis of yoghurt on 21st day of storage period**Table 4. Microbial analysis of three samples T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> after 21 days of storage at 4-6 °C temperature.

Sample & Dilution	Yeast(CFU/ml)	Mould (CFU/ml)	Bacteria (CFU/ml)
T <sub>1</sub>			
10 <sup>-1</sup>	20	Nil	25
10 <sup>-2</sup>	22x10 <sup>2</sup>	22x10 <sup>2</sup>	22x10 <sup>2</sup>
10 <sup>-3</sup>	19x10 <sup>3</sup>	27x10 <sup>3</sup>	24x10 <sup>3</sup>
10 <sup>-4</sup>	24x10 <sup>4</sup>	20x10 <sup>4</sup>	29x10 <sup>4</sup>
10 <sup>-5</sup>	25x10 <sup>5</sup>	26x10 <sup>5</sup>	28x10 <sup>5</sup>
Sample & Dilution	Yeast(CFU/ml)	Mould (CFU/ml)	Bacteria (CFU/ml)
T <sub>2</sub>			
10 <sup>-1</sup>	24	23	25
10 <sup>-2</sup>	27x10 <sup>2</sup>	28x10 <sup>2</sup>	26x10 <sup>2</sup>
10 <sup>-3</sup>	30x10 <sup>3</sup>	32x10 <sup>3</sup>	28x10 <sup>3</sup>
10 <sup>-4</sup>	29x10 <sup>4</sup>	29x10 <sup>4</sup>	27x10 <sup>4</sup>
10 <sup>-5</sup>	28x10 <sup>5</sup>	29x10 <sup>5</sup>	30x10 <sup>5</sup>
Sample & Dilution	Yeast(CFU/ml)	Mould (CFU/ml)	Bacteria (CFU/ml)
T <sub>3</sub>			
10 <sup>-1</sup>	Nil	Nil	Nil
10 <sup>-2</sup>	Nil	Nil	Nil
10 <sup>-3</sup>	Nil	Nil	Nil
10 <sup>-4</sup>	15x10 <sup>4</sup>	Nil	Nil
10 <sup>-5</sup>	18x10 <sup>5</sup>	Nil	21x10 <sup>5</sup>

Results in Table 3 indicates that there was no growth of mould and bacterial count in T<sub>3</sub> sample but in one dilution very less growth of yeast was observed in T<sub>1</sub> sample. Results in Table 4 indicates that there was more microbial growth in T<sub>1</sub> and T<sub>2</sub> samples which showed a significant difference and is not safe for consumption. There was also less microbial growth observed in T<sub>3</sub> sample and this count was less than generally regarded as safe value. So the product formulated with suitable proportion of live starter culture is less prone to microbial contamination in 21 days of storage at 4-6°C. Some researchers suggest concentration levels superior to 10-6cfu/ml, other agree concentrations superior to 10-7cfu/ml and 10-8cfu/ml as satisfactory levels (Lourens-Hattingh and Viljeon, 2001).

**Organoleptic Evaluation:**

The acceptance of a product will depend on whether it responds to consumer needs and on the degree of satisfaction it is able to provide. For this reason, consumers' opinions must be taken into consideration, not only to evaluate the acceptance of the final product, but also from the beginning of the process of product development. The organoleptic evaluation is done for the product acceptability by the consumers with the help of a score card with specific attributes to know the consumer ranking between three samples T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. Yoghurt should be firm, free from any whey separation and have a creamy layer. Generally the appearance of yoghurt should convey smooth, homogenous, moderately firm gel or custard like body and texture and a uniform off white color.

Table5. Mean Scores for various attributes

Attribute	T1	T2	T3
Colour	4.43±1.2	4.1±0.34	4.18 ±1.47
Flavour	3±1.63	4.12±1.58	4.47±0.88
Appearance	4.3±1.24	4.43±1.2	4.37±1.36
Texture	4.18±0.98	3.87±1.4	4.56 ±0.81
Acceptability	2.5±1.03	3.37±1.54	4.87 ±0.34

Table5. Gives scores obtained in organoleptic evaluation with respect to different attributes. Values are represented as Mean±S.D. Results of the values from the table 5 indicate that in colour T<sub>1</sub> (Control) was more attractive. It is the sample incorporated with commercial market plain yoghurt. In appearance aspect T<sub>2</sub> sample scored the most. In the flavor attribute T<sub>3</sub> sample scored high showing a significant difference compared with T<sub>1</sub> and T<sub>2</sub>, this might be due to the natural probiotic incorporation which develops the good components mainly lactate, aroma compounds (acetaldehyde, acetone and diacetyl) and Exopolysaccharides. In texture T<sub>3</sub> sample had a good firm texture as an appropriate amount of sweet potato was added as a stabilizer, it also has a pleasant mouth feel. Low fat yoghurts are known for poor solid contents, which make them susceptible to syneresis unless they are heavily stabilized (Trachoo and Mistry, 1998). The sample T<sub>3</sub> scored highest in final overall acceptability as it had correct composition, good taste and excellent acceptability. Sensory analysis revealed that FOS addition reduced the perception of sourness and increased sweetness.





Plate1. Sample T1  
(Plain yoghurt + Sweet

Plate.2. Sample T2  
(Plain yoghurt+FOS  
Potato)

Plate 3. Sample T3 (Live  
active culture + FOS +Sweet potato)

Out of all three formulations made, T3 sample proved to be the best product as seen from the results of analysis of quality parameters, maintaining the CFU count until the end of the storage period, making it safe for consumption. Sensory scores were also higher and it was the best acceptable product by consumers. So the proportion of 30% sweet potato used, with live active starter culture at 3% in T3 sample and use of proper quality control measures made it as an acceptable product. About 15 elderly people both male and female were given the product T3 which had scored highly in the sensory evaluation, and their reaction and opinion was obtained informally. The product was extremely acceptable to each and every individual and most of them expressed the opinion that they would purchase and consume it if it is available. The accepted product T3 sample incorporated with probiotic and prebiotic in combination of sweet potato was a healthy nutritious product in treating certain digestive disorders suitable for all age groups especially to the elderly as more often gastric discomfort was observed in that age group. Prevention of all these problems can be solved by the consumption of symbiotic yoghurt regularly. Availability of such symbiotic yoghurt in the market can help people of all ages not only to enjoy its excellent taste but also reap the health benefits provided by it.

#### IV. Conclusion:

In the present project study, symbiotic yoghurt (Combination of Probiotic and prebiotics) product development, physico chemical and nutritional analysis, consumer acceptability was done. Most of the early developments were done with yoghurt with different types of fortifications with folic acid and iron incorporations, carrot yoghurt, pomegranate yoghurt, carbonated yoghurt, banana yoghurt etc. But the present study focussed on Symbiotic yoghurt, with sweet potato incorporation for additional calorie content and as a stabilizer in the product was used. So in the research work product was formulated and standardized with live probiotics. The product formulations were done in three combinations with different proportions of ingredients. T1 (Plain yoghurt + sweet potato), T2 (Plain Yoghurt + FOS + sweet potato), T3 (Live Active Culture 3% + sweet potato). Double toned milk was used for the development of low fat yoghurt. The milk was homogenized, pasteurized and stabilized with boiled and mashed sweet potato divided into three samples with different formulations. The respective formulations are made and kept in incubator for 3 hours, at 41 – 42 °C and immediately transferred to refrigerator, stored at 4 °C for further analysis. In the evaluation the physico chemical analysis, sensory evaluation with semi trained panelists, Shelf life study of the product were done. For three samples T1, T2 and T3, analysis concluded that Percentage of Total solids was less for T3 sample this is yoghurt prepared with live active culture, as double toned milk was used in the process when compared with other two samples. The percent of SNF was also less for T3 sample when compared with T1 and T2. The titratable acidity for T3 sample was less and it indicates percentage of lactic acid formation which is again based on the rate of fermentation. The sample T3 contains live active culture over acidification did not occur when prepared with proper quality measures, for other two samples acidification was more it is the composition of plain yoghurt incorporated sample. The protein content in T3 sample was also high due to higher percentage of sweet potato compared with other two samples and also the symbiotic combination of the yoghurt gives a complete protein with the mutual supplementation of amino acids. The CHO content was also more for T3 sample as it has more percentage of sweet potato which adds to the calorie content compared with other two samples. The fat percent was less for T3 sample as its composition includes double toned milk for the development of low fat yoghurt while other two samples are prepared with outside full fat plain yoghurt. There was not that much difference in mineral availability of calcium and phosphorous in all three samples. There was no significant difference in pH of all three samples but after three weeks of storage for T1 and T2 sample the values were decreased due to rapid growth and acidification but for T3 sample which was with live active probiotics there was no difference after 21 days of storage also. The results from sensory analysis indicate that the color of T1 was more attractive as it is the sample with commercial outside market yoghurt. In the flavor attribute T3 sample was better due to the natural probiotic incorporation which develops good components mainly lactate, aroma compounds

(acetaldehyde, acetone and diacetyl) and exopolysaccharides. In appearance aspect T2 sample scored more. In texture T3 sample has good firm texture and with appropriate amount of sweet potato as a stabilizer, also has a pleasant mouth feel. The final overall acceptability scored highest for T3 sample with correct composition and good taste. Results in the microbial analysis on first day of yoghurt indicates there was no growth of yeast, mould and bacterial count but in one dilution very less growth of yeast is observed in T1 sample. Then after 21 days of storage indicates that there was more microbial growth in T1 and T2 samples, make them unsafe for consumption. However, there was also less microbial growth observed in T3 sample as this count was less than generally regarded as safe value (GRAS). So the product formulated with suitable proportion of live starter culture was less prone to microbial contamination in 21 days of storage. Stabilizers added to the coagulum must be of excellent microbiological quality, otherwise the shelf life of the product could be reduced. The yoghurt prepared with live active probiotics may be considered as good quality yoghurt, in proper phase of fermentation, acid formation, maintaining optimum pH of product. With the incorporation of Fructo-oligosaccharide there is good water holding capacity, in sensory analysis also the FOS incorporated product has good taste and smooth mouth feel. The sample T3 made with live starter probiotic shows the benefits of a symbiotic combination. This is indicated by pleasant appearance, good flavor, overall acceptability was also best for T3 sample made out of live starter probiotics. The stabilizer sweet potato incorporated with 30% level gives the better viscosity as the molecules of a stabilizer are capable of forming a network of linkages between the milk constituents mainly proteins, it also retards water forming in product, and increases calorie content of product. The future of yogurt manufacturing is focusing on the development of new flavors and longer lasting yogurts for satisfying the consumer desires, the suppliers of the bacterial cultures are conducting research should also concentrate on development of uniquely flavored yogurts.

### Suggestions for future research

- More research need to be done for evidence of health benefits beneficial to all ages of humans.
- Quality control measures need to be designed to maintain microbial quality, bacterial culture propagation, milk quality testing immediately after receiving, degree of pasteurization also need to be measured after pasteurization.
- Development technologies in yoghurt preparation which could reduce energy consumption by minimizing refrigeration.
- Development of probiotic yoghurts in different flavours.

### References

- [1]. Agrawal, R. 2005. Probiotics: an Emerging Food Supplement with Health Benefits. *Food Biotechnology*. 19: 227-246.
- [2]. Andersson, H., Bruce, A., Roos, S., Wadstrom, T and Wold, A.E. 2001. Health effects of probiotics and prebiotics: A literature review on human studies. *Scandinavian Journal of Nutrition*. 45: 58-75.
- [3]. AOAC (Association of Official Analytical Chemists). In Williams, S (Ed.), *Official methods of analysis of AOAC International*. Arlington: AOAC International. 2002.
- [4]. Bojgaard, S.E. 1987. XXII International Dairy Congress. IE. 259.
- [5]. Carr, B. T., Meilgaard, M. and Civille, G. V. *Sensory evaluation techniques*. Washington, DC: CRC Press. 1999.
- [6]. Dempsters. 2007. Introducing fibre with a difference, a prebiotic difference Stephen Olmstead, M.D., David Wolfson, N.D., Dennis Meiss, Ph.D and Janet Ralston, B.S. 2008. Klaire labs™ A division of Prothera, inc. 1-8.
- [7]. Donini, L.M, Savina, C and Cannella, C. 2009. "Nutrition in the elderly – the role of fiber". *Archives of Gerontology Geriatrics*. Supplement 1: 61-69.
- [8]. Fuller, R. 1989. Probiotics in man and animals: A review. *Journal of Applied Bacteriology*. 66 (5): 365-368.
- [9]. Guyonnet, D., Chassany, O and Ducrotte, P. 2007. "Effect of a fermented milk containing *Bifidobacterium animalis* DN 173 010 on health related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomised, double blind, controlled trial". *Alimentary Pharmacology and Therapeutics*. 24: 475-486.
- [10]. IDF. 1983. Yoghurt: Enumeration of characteristic microorganisms colony count technique at 37°C. IDF Standard 117 International Dairy Federation. Brussels.
- [11]. Lewis, S., Burmeister, S and Brazier, J. 2005. Effect of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhea: a randomized, controlled study. *Clinical Gastroenterology and Hepatology*. 3: 442-448.
- [12]. Loessner, M., Guenther, S., Steffan, S and Scherer, S. 2003. A pedocin-producing *Lactobacillus plantarum* in a multispecies cheese surface microbial ripening consortium. *Applied Environmental Microbiology*. 69:1854-1857.
- [13]. Lourens-Hattingh, A and Viljoen, B.C. 2001. Review: Yoghurt as probiotic carrier in food. *International Dairy Journal*. 11: 1-17.
- [14]. Oliveira, R.P., Perego, De S., P., Converti, P and De Oliveira, M. N. 2009a. Growth and acidification performance of probiotics in pure culture and co-culture with *Streptococcus thermophilus*: The effect of inulin. *LWT-Food Science and Technology*. 42:1015-1021.
- [15]. Roberfroid, M..B., Van Loo, J. A and Gibson, G..R .1998. The bifidogeni nature of chicoryinulin and its hydrolysis products. *Journal of Nutrition*. 128 (1):11-19.
- [16]. Russell, R.M. 1992. Changes in gastrointestinal function attributed to aging. *American Journal of Clinical Nutrition*. 55(6): 1203S-1207S.
- [17]. Schiffrin Eduardo, J., Parlesak Alexandr., Bode Christiane., Bode Christian. J., van't Hof Martin, A., Grathwohl Dominik and Guigoz Yves. 2009. Probiotic yoghurt in the elderly with intestinal bacterial overgrowth: endotoxaemia and innate immune functions. *British Journal of Nutrition*. 101: 961-966.
- [18]. Stephen Olmstead, M.D., David Wolfson, N.D., Dennis Meiss, Ph.D and Janet Ralston, B.S. 2008. Klaire labs™ A division of Prothera, inc. 1-8.
- [19]. Tamime, A.Y. and Deeth, H. 1980. Yogurt: Technology and biochemistry. *Journal of Food Protection*. 43:939-977.
- [20]. Tamime, A.Y. and Robinson, R.K. 1999. *Yoghurt: Science and Technology*. 2nd edn. CRC Press, Boca Raton, Florida.
- [21]. Vouloumanou, E.K., Makris, G.C., Karageorgopoulos, D.E and Falagas, M.E. 2009. Probiotics for the prevention of respiratory tract infections: a systematic review. *International Journal of Antimicrobial Agents*. 34(3): 197.e1-10. Jan 28.