PROBIOTIC PROFILING AND ORGANOLEPTIC EVALUATION OF TRADITIONAL CEREAL BASED FERMENTED DRINK AND ITS MARKET POTENTIAL

Synopsis of PhD Thesis

A report submitted in partial fulfillment for the

Degree of Doctor of Philosophy

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CHAPTER 1
INTRODUCTION

The Indian food and beverage industry has witnessed drastic changes in recent years. Healthier product alternatives, including beverages specifically designed to provide energy, nutrition and health to the human body, are becoming top priority *(Federation of Indian Chambers of Commerce & Industry, 2015)*. The desire for health and concern for ill-health has driven consumers towards these beverages. Healthier product alternatives, including beverages specifically designed to provide energy, nutrition and health to the human body, are among becoming top priority. The consumers for health beverages are people from the different walks of life but majority of them come from the younger and older generations.

The rise in health-consciousness is also reflected in the growth of health care spending, which is growing at a Compound Annual Growth Rate (CAGR) of 14 percent, from 5.5 percent of the GDP in 2009 to 8 percent in 2012. The figure is expected to grow to US$ 280 billion by 2020. Concomitantly, the market for health beverages is beginning to pick up speed and volume. There is a huge opportunity in the packaged beverage market being fuelled by a rise in disposable incomes, changing lifestyles and a burgeoning younger middle class priority *(Federation of Indian Chambers of Commerce & Industry, 2015)*..

1.1.Health beverages in India

In spite of all the health awareness among the population, the availability of health beverages in Indian markets is still scarce. The Indian market for beverages has been segmented on the basis of types of products that is carbonated and non- carbonated. Carbonated products account for nearly 61-62% of the total soft beverages market. The brands that fall in this category are Pepsi, Coca-Cola, Parle, etc. Non- carbonated segment which constitutes 36% can be divided into 2 categories that is dairy based products like *lassi*, curd, flavored milk, buttermilk etc and fruit based on the

types of flavors available like orange, lime, lemon and mango, etc. Due to the trend of health-consciousness of consumers, the Indian noncarbonated beverage segment has become one of the fastest growing businesses currently valued at Rs 20,000 crore *(FnB news, 2013).* It is likely to grow at a healthy CAGR of 35 per cent, and touch 54,000 crore by 2018 *(Associated Chambers of Commerce and Industry of India, 2013)*

According to the *Euromonitor International report (2014),* Indian consumers are spending more and more on nutritional supplements to combat the effects of health problems. This indicates that people are concerned about their health. Flavored milk, energy beverages, liquid-meal replacements, weight-directed products, ready-to beverage meals and functional beverages could act as substitute of carbonated beverages in Indian market. The non-carbonated beverage industry has tremendous potential for future developments in India.

1.2.Consumption pattern of packaged beverages

Recent trends in final food demand show that the concept of food has undergone a radical transformation. In addition to the nutritional and sensory properties of food, consumers are also well aware about their important role in the maintenance of health, on psycho-physical well-being and prevention of certain diseases. Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers *(Azzurra and Paola, 2009).*

Significant predictors of functional food consumption are related to consumers' health motivation, perceived diet effectiveness of products, and knowledge about nutrition. There is evidence that consumers most likely to have a positive attitude toward functional foods are the ones who have faced illness among relatives or have experienced illness themselves *(Dowd and Burke, 2013).* There is no doubt that the functional food industry is growing steadily worldwide. However,

successful commercialization of new products is proving to be challenging especially due to the need for a strategic approach to product development processes *(Shamal et al, 2017).*

Increasing urbanisation, growing awareness and affluence continued to drive consumers to make healthy choices and hence, increasingly switch to packaged beverage milk products over the review period. Which is expected to grow by Compound Annual Growth Rate (CAGR) 7% in the coming years.

In today's modern world, due to urbanization, the change in dietary habits and lifestyle pattern has itself forced the health conscious consumers to search for an alternative treatment regime for various ailments caused by lifestyle related diseases. In this direction, probiotics and probiotic based food supplementation have drawn interest of consumers *(Parvez et al, 2006; Raguvanshi et al, 2015).* The consumption of probiotics exerts myriad of beneficial effects which is evidenced by rigorous scientific evaluation. It is a major focus of attention of scientists across the world due to their promising health benefits and their applications offers an innovative approach for development of novel probiotic formulations *(Bagchi, 2014).*

The proposed fermented beverage is a modification of the traditional fermented beverage which has been used in India since ages. Commercialization of this beverage will help easy access of probiotics to the entire population. Consumption of this fermented beverage will be beneficial for all the age groups and will help to:

- Improve bowel movement and aid digestion
- Maintain balance of good and bad bacteria
- Reduce toxins in our body
- Help build the immune system (reduce risk of infections)

The technological application of the proposed fermented beverage aims to combine the potential health benefits of the good bacteria with their ability to grow in with cereals, resulting in a nutritionally healthy and desirable product for the consumers. Another important fact is the increasing demand for consumer convenience. Present day consumers prefer foods that promote good health and prevent disease. Furthermore, these foods must fit into current lifestyles providing convenience of use, good flavor, and an acceptable price value ratio. Such foods constitute current and future waves in the evolution of the food development cycle. Development and marketing of this fermented beverage will not only conserve the traditional fermented beverage but will also be a ready to consume probiotic product for the consumers readily available in the markets.

1.3.Indigenous fermented foods

1.3.1. International status

Indigenous fermented foods have been prepared and consumed for thousands of years, and are strongly linked to culture and tradition. The traditional fermented foods are mainly obtained from dairy products viz. yoghurt, dahi, kefir, cheese (after long storage), and fermented vegetable or from vegetable juices and from non-fermented fruit and berry juices. Amongst Asian countries, the most prominent ones which consume probiotics are Japan, Korea, Indonesia, India, Nepal, Sri Lanka. Reports of fermented foods such as Kefir (Russia), Calpis (Japan), Tofu (Southeast Asia), Kimchi (Korea), Kaymak (Turkey) which are used for health benefits, characteristic of various societies have existed in the literature *(Tamang and Kailasapathy, 2010).*

It is, therefore, not surprising that fermented foods and beverages make a big contribution to people's diets worldwide *(Sanni, 1993).* It is reported that fermented foods globally contribute 20 to 40% of the food supply and usually, a third of the food consumed by man is fermented

(Campbell-Platt, 1994). This renders fermented foods and beverages a significant component of people's diets globally.

1.3.2. National status

In the Indian subcontinent, fermented food and beverages, prepared using local food crops and other biological resources have been going on since time immemorial and is a common practice even today *(Roy et al., 2004). Idli, dosa, dhokla, khaman, handva*, etc are certain popular traditional fermented foods which are consumed throughout India, particularly in southern and western parts of India. A variety of region specific fermented foods and beverages are traditionally produced and consumed, and even locally marketed in North-East India *(Das and Deka, 2012).* At certain times, sour buttermilk or yeast is added in order to enhance fermentation.

In eastern Himalayan regions of Darjeeling hills and Sikkim, *Kinema*, a product of fermented soybean is consumed *(Nagai and Tamang, 2010).* In certain parts of India, fermented rice was mixed with buttermilk and salt for direct consumption. The *jalebies* and *kanji* are mainly consumed in northern India *(Sekar and Mariappam, 2007).* Amongst the dairy products, *dahi*, *rabdi*, *paneer*, *shrikhand*, *misti dahi* and *chhurpi*, are used in northern, western, eastern and Himalayan region of India, respectively *(Nehal, 2013).*

1.4.Importance of Fructooligosaccharide (FOS) as a prebiotic

FOS has been recognized for its great potential which is becoming apparent as a considerable food element in increasing the bacterial bionomics which might have a concrete role in reducing the burden of CVDs. Many recent researches have documented that consumption of FOS reduces the incidences CVDs by regulating hypertension, improving glycemic, lipemic control and reducing

the expression of inflammatory markers *(Ooi and Liong, 2010, Dehghan et al 2014, Asemi et al, 2013).* FOS supplementation in pre hypertensive type 2 NIDDM subjects have also lead to significant reduction in Fasting Blood Sugar (FBS), Post prandial blood glucose(PP2BS) and glycated haemoglobin by 6.3%, 9.8% and 10.6% respectively. Another study conducted with FOS added milk revealed significant reduction of 5.2% and 7.8% for FBS and PP2BS respectively *(Parnami et al, 2011).* Studies have also documented significant reduction in TG, TC, LDL-C, VLDL and increase in HDL-C with consumption of FOS *(Sheth et al, 2015).* Significant reduction in ha-CRP levels and BMI had also been reported in many studies *(Sheth and Asudani, 2014; Jain and Sheth 2014).* FOS is now being considered worldwide and has a great potential for future. Many international industries and organizations are now coming up with new and innovative products using FOS.

The proposed research study is related to a fermented product development using a prebiotic FOS, studying its probiotic and market potential and its commercialization, so that it is available to the population.

1.5.**Rationale for the proposed work**

India has a rich culture of consuming milk and several fermented milk based products since ancient civilizations. Some of these are slowly becoming extinct and lesser known to the new generations. These fermented products can be reinvented by applying modern technologies for mass production to meet the consumer's new demand in response to changing lifestyle.

Ambil is an indigenous natural cereal based lactic fermented beverage traditionally from Maharashtra. So far the process of its preparation remains a household art, which may lead to wide variation in its final quality and short shelf life.

No work of scientific significance has been reported in literature, on its probiotic properties, standardization and packaging, acceptability and feasibility of its modification in terms of base ingredients and flavors. The market potential also needs to be studied and established through consumer acceptability trials.

1.6.Hypothesis

Null hypothesis

1. *Ambil* does not contain probiotic organisms
2. *Ambil* with added FOS does not have high sensory qualities and acceptability
3. *Ambil* does not have good consumer acceptance and market potential

Alternate hypothesis

1. *Ambil* contains probiotic organisms
2. *Ambil* with added FOS have high sensory qualities and acceptability
3. *Ambil* have good consumer acceptance and market potential

1.7.Objectives

Broad Objective

Development of cereal buttermilk based fermented beverage and studying its probiotic profile, organoleptic qualities, consumer acceptability and market potential

Specific objectives:

1. To standardize the *Ambils* using four different cereals, buttermilk and FOS and study their sensory qualities
2. To study the probiotic profile of buttermilk and standardized *Ambil*s in terms of strains of *Lactobacilli* and *Bifidobacteria*

3. To determine the nutritional profile and storage stability of fresh and packaged *Ambils* in terms of its chemical and microbial qualities
4. To study the consumer acceptability and market potential of the most acceptable *Ambil*

In order to fulfil the above objectives, the study was divided into four phase as follows:

Phase I – Standardization of *Ambils* using four different cereals, buttermilk and FOS and study their sensory qualities

(i) Standardization of *ambil* using the traditional ingredients including rice and buttermilk.

(ii) Cereal and flavor based modification of *ambil*s.

(iii)Assessment of organoleptic properties and overall acceptability of developed products.

Phase II – Probiotic profiling of buttermilk and standardized *Ambil*s in terms of strains of *Lactobacilli* and *Bifidobacteria*

(i) Probiotic profile for the following species

- *Bifidobacterium bifidum, B. lactis, B. logum and B. adolescentis*
- *Lactobacillus rhamnosus, L. reuteri, L. casei and L. acidophilus*

Phase III – Determination of nutritional profile and storage stability of fresh and packaged *Ambils* in terms of its chemical and microbial qualities

(i) Nutritional analysis of buttermilk and the fresh *ambil*s.

(ii) Tetra packaging and storage of the most acceptable *ambil*

(iii) Storage stability of fresh and the tetra packed packaged *ambil*.

Phase IV – Determination of Consumer acceptability and market potential of the most acceptable *Ambil*

(i) Selection of consumers by convenient sampling technique.

(ii) Defining five age groups, elderlies, adults, adolescents, school going children and pre-schoolers.

(iii) Determination of organoleptic properties and consumer acceptability of developed products

(iv) Studying the market potential using a feedback form with questions related to the packaged product.

Phase V- Data analysis and report writing

CHAPTER 2

REVIEW OF LITERATURE

This chapter will focus on the available literature under following heads:

- Beverage industry in nutshell
- Consumption pattern of beverages worldwide
- Consumer demand for health beverages
- Fermentation as a technology in health beverages
  - History of fermentation
  - Fermentation process
  - Physicochemical changes
  - Functional properties of micro organisms in fermented foods
  - Fermented foods – global trends and consumption pattern
  - Indian scenario

- Use of probiotics and prebiotics in foods
- Use of probiotics and prebiotics in health beverages
- Fructooligosaccharide (FOS) as a prebiotic
- FOS - its types and technological function
- FOS in foods
- Storage stability of foods containing probiotics and prebiotics
- Commercialization of health beverages

CHAPTER 3

METHODS AND MATERIALS

The present study was undertaken under four phases-

3.1. Phase 1 - Standardization of *Ambils* using four different cereals, buttermilk and FOS and study their sensory qualities

(i) Standardization was carried out for all the *ambil*s using the standard recipes (*Ramakrishnan 1977; 1979*)

(ii) Cereal and flavors based modification were carried out for *ambil* using rice, pearl millet, barley and finger millet.

(iii) Addition of four different flavors to the *ambil*s namely rose, *khus*, chocolate and salt-cumin.

Ambil was developed using cereals (8%), buttermilk (38%) and water (46.5%) using standard methods (*Ramakrishnan 1977; 1979*). Colours (0.3%) and essences (0.3%) were added to develop three sweet flavors rose, khus, chocolate and one salt - cumin

flavor using 0.4% and 0.6% of salt and cumin respectively. The levels of these ingredients were decided on the basis of preliminary trials. FOS (6.5%) was added to all the flavors of *ambil*s and stirred well. The final product was cooled to 5 °C and stored in refrigerator (5–7 °C).

(iv) Organoleptic evaluation of the standard and FOS incorporated products was conducted by 25 semi trained judges, after

a. Screening of panelists by Threshold test (Ranganna, 2010)

b. Development of composite score cards for the sensory evaluation of the products

3.1.1. Tools for Organoleptic evaluation of the products

Composite score analysis: Organoleptic evaluation was carried out on the *ambil* samples containing different flavors. Twenty five semi trained panellists were selected using the sensitivity threshold test. The panel members were asked to rate the samples for taste, appearance/colour, after taste, absence of defects, consistency, and overall acceptability using a composite score analysis in triplicates (Rangana, 2010).

3.1.2. Statistical analysis

Data analysis was conducted using Statistical Package for the Social Sciences (SPSS – 16 version). The data was statistically tested using analysis of variance (one-way ANOVA) to determine if statistical difference ($p<0.05$) existed, and followed by Fishers Least Significant Difference (LSD) test to identify statistical differences among the means. Triplicate measurements were taken for each analysis. Results from all the tests were expressed as means ± standard deviations (SD).

3.2. Phase II – Probiotic profile of buttermilk and standardized *Ambil*s in terms of strains of *Lactobacilli* and *Bifidobacteria*

3.2.1. Specificity of primers

Novel species-specific primers for identification of the target species of Lactobacillus and Bifidobacterium were designed from 16S rRNA, 16S-23S rRNA intergenic spacer region, and 23S rRNA sequences. The specificity of the species-specific primer pairs was evaluated by Polymerase Chain Reaction (PCR) with genomic DNAs purified from the reference strains.

3.2.2. DNA isolation and sequencing

Freshly prepared fermented beverage, *Ambil* was subjected to serial dilutions (10 folds), and was plated on MRS broth. The Petri plates were incubated for 72hrs at 37°C. Once the bacterial colonies developed, they were transferred to 200µl of PCR water and was mixed thoroughly, and subjected to DNA isolation, followed by PCR amplification and gel electrophoresis. The sequencing of the amplified DNA fragment was carried out with an ABI PRISM 3700 DNA analyser. The DNA sequences were analysed using the Vector NTI software Version 7.

3.3. Phase III – Determination of nutritional profile and storage stability of fresh and packaged *Ambils* in terms of its chemical and microbial qualities

3.3.1. Compositional analysis of buttermilk and fresh *ambil*s (Rangana, 2010).

i. Proximate constituents
ii. Vitamins
iii. Minerals
iv. Microbial analysis for TPC, Coliform, yeast and molds

3.3.2. Tetra packaging and storage of the most acceptable *ambil*

Raw material was procured and was transported to Amul Fed Mother dairy, Ahmedabad for bulk production and packaging. Rice *ambil* was prepared using standard procedure, and was subjected to homogenization and was tetrapacked (135 to 150°C for 3 to 5 seconds).

3.3.3. Compositional analysis packaged rice *ambil* was done for: (Rangana, 2010).

i. Proximate constituents

ii. Vitamins

iii. Minerals

3.3.4. Storage stability of fresh and the tetra packaged *ambil*.

i. Storage stability of the fresh *ambil* was studied for 5 days consecutively for viscosity, pH, peroxide value and sensory evaluation, by semi trained panel members from 0 to 5 days.

ii. Microbial quality of the developed products by estimating Total Plate Count, coliforms and yeast and mold from 0 to 5 days.

iii. Storage stability of the packaged rice *ambil* was studied for a period of 6 months for viscosity, pH, peroxide value and sensory evaluation, by semi trained panel members at 0 day, 30^{th} day, 60^{th} day, 90^{th} day, 120 days, 150 days and 180 days (6 months)

3.3.5. Tools for Organoleptic evaluation of the products

Composite score analysis: Organoleptic evaluation was carried out on the fresh and packaged rice *ambil* samples. Ten semi trained panellists were selected using the sensitivity threshold test. The panel members were asked to rate the samples for taste, appearance/colour, after taste, absence of defects, consistency, and overall acceptability using a composite score analysis in triplicates.

3.3.6. Statistical analysis

Data analysis was conducted using Statistical Package for the Social Sciences (SPSS – 16 version). The data was statistically tested using analysis of variance (one-way ANOVA) to determine if statistical difference ($p<0.05$) existed, and followed by Fishers Least Significant Difference (LSD) test to identify statistical differences among the means. Triplicate measurements were taken for each analysis. Results from all the tests were expressed as means ± standard deviations (SD).

3.4. Phase IV - Determination of Consumer acceptability and market potential of the most acceptable *Ambil*

3.4.1. Consumer acceptability

Five age groups were defined which included elderlies, adults, adolescents, school going children and pre-schoolers. The consumers were selected by convenient sampling technique from a Food-fest organized at Faculty of Family and Community Sciences, Vadodara, Experimental School of The MS University, Vadodara, Old age homes and a local supermarket of Vadodara, depending on the subject's willingness to taste the product. A total of 518 subjects were selected using the percentage census of population. The consumer acceptability trials were continued until the target number of consumers from each group was reached.

3.4.2. Market potential

Market potential was studied on the same subjects excluding the age group of less than 10 years (n=429), using a feedback form. Questions were asked regarding their willingness to purchase the same beverage if available in the market, preference in any

different flavor, package size preferred, packaging material and cost expected if introduced commercially in supermarkets of Vadodara.

Selection of subjects and Sample size calculation:

Sample size was calculated based on the population census of Gujarat *(Census of India, 2011; Directorate of Economics and Statistics Government of Gujarat, 2011)* for all the different age groups. All the subjects selected were a representative of the total percentage population of that age group *(Census of India, 2011; Directorate of Economics and Statistics Government of Gujarat, 2011).*

Inclusion criteria:

- Elderlies (>65 years)
- Adults (20-64 years)
- Adolescents (10-19 years)
- School going children (6-10 years)
- Preschool children (3-5 years)

Exclusion criteria: Children below 3 years of age.

3.4.3. Tool used

Questionnaire which included questions regarding their willingness to purchase the same beverage if available in the market, will they like to purchase the same product in any different flavor, quantity of beverage and its cost and packaging material and cost of the product

3.4.4. Statistical analysis

Statistical analysis was performed using Microsoft excel, 2010 software. Results were expressed as mean values ±SD for all the four products.

Phase V- Data analysis and report writing

CHAPTER 4

RESULTS

The results of the present study entitled "Probiotic profiling and organoleptic evaluation of cereal buttermilk based fermented beverage and its market potential" are presented in the following sections:

Phase I – Standardization of *Ambils* using four different cereals, buttermilk and FOS and study their sensory qualities

Phase II – Probiotic profiling of buttermilk and standardized *Ambil*s in terms of strains of *Lactobacilli* and *Bifidobacteria*

Phase III – Determination of nutritional profile and storage stability of fresh and packaged *Ambils* in terms of its chemical and microbial qualities

Phase IV – Determination of Consumer acceptability and market potential of the most acceptable *Ambil*

4.1. Phase I – Standardization of *Ambils* using four different cereals, buttermilk and FOS and study their sensory qualities

4.1.1. Assessment of organoleptic properties through composite scoring for FOS added rice ambil with different flavors

The organoleptic scores of rice *ambil* with different flavors varied significantly with different flavors and colors.

Color and appearance attributes of the rice *ambil*s varied significantly with different flavors and colors. Rose flavor was the most acceptable, followed by khus (9.19) and salt-cumin (9.04). The color of chocolate (8.89) flavored *ambil* had the least acceptability. Non-significant increase in the consistency scores was reported in the different flavors of rice *ambil*s. The acceptability in terms of taste and flavor varied significantly ranging from 25.64 to 27.47. Maximum score was obtained by salt-cumin (27.47) flavored rice *ambil*, followed by rose (26.92), khus (25.71) and chocolate (25.64).

Mouthfeel of the *ambil*s also showed significant difference. Maximum score was obtained by salt-cumin (8.97) *ambil*. Slight reduction in the scores was observed from rose (8.74), chocolate (8.62), and khus (8.42) flavors. The scores for after taste ranged from 8.32 to 8.88, with a significant difference amongst all the flavors. In spite of this difference, all

products were in acceptable range and thus it can be concluded that none of these beverages had shown any unacceptable after taste.

Overall acceptability showed a significant difference ($p<0.000$) with salt-cumin (18.17) flavor having the highest acceptability, preceded by chocolate (17.11) and rose (17.69). Khus flavor scored the minimum with 16.69, depicting that it was least liked by the panel members.

4.1.2. Assessment of organoleptic properties through composite scoring for FOS added barley ambil with different flavors

The organoleptic scores of barley *ambil* with different flavors varied significantly with different flavors and colors.

Considering the color and appearance of all the *ambil*s, no significant difference was found between color, indicating that all were equally acceptable by the panel. The scores ranged from 8.64 for chocolate flavor followed by salt-cumin (8.75), khus (8.90) and rose (9.00). The choice of taste and flavor varied significantly. Maximum acceptability was found for salt-cumin (25.64) flavored barley *ambil*, followed by rose (24.32) and chocolate (24.07) and least by khus (23.75).

Mouthfeel of the product were also significantly different. Salt-cumin (8.47) had the highest score for good mouth feel, followed by chocolate (7.99), rose (7.79) and khus (7.67). Significant difference in the after taste of the different flavors was observed. Maximum scores obtained by salt-cumin (8.60), depicting that salt cumin is the most acceptable and had shown minimum after taste. Chocolate (8.12), rose (8.06) and khus (8.01) were also acceptable and had shown a slight after taste as compared to salt-cumin flavor.

Overall acceptability showed a significant difference with salt-cumin (17.51) flavor having the highest acceptability, followed by chocolate (16.18), rose (16.03) and khus (15.86).

4.1.3. Assessment of organoleptic properties through composite scoring for FOS added pearl millet ambil with different flavors

The organoleptic scores of pearl millet *ambil* with different flavors varied significantly with different flavors and colors.

The color and appearance of the pearl millet *ambil*s, varied significantly with the colors used, ranging from 8.49 (chocolate flavor) to 9.04 (rose flavor). Pink color of rose *ambil* was more appealing to the panel members as compared to others, thus confirming the best acceptability. A non-significant increase in the consistency scores was reported in the different flavors of pearl millet *ambil*s.

The choice of taste and flavor also depicted significant difference ($p<0.001$). Maximum acceptability was found for salt-cumin (26.07) flavored pearl millet *ambil*, followed by rose (25.36), khus (24.1) and chocolate (24.08).

Significant increase in the mouthfeel of the different flavors was observed. Salt-cumin (8.38) had the highest score for good mouth feel, followed by rose (8.25) and khus (7.92). Least was scored by chocolate flavored *ambil* (7.90). The after taste of the different flavors was observed in the range of 7.76 to 8.38. Maximum scores obtained by salt-cumin (8.38) flavor followed by rose (8.08), chocolate (7.81) and khus (7.76). None of the flavors of pearl millet *ambil* had shown any defects and were all acceptable by the panel members.

Overall acceptability showed a significant difference ($p<0.000$) with salt-cumin flavor (17.49) having the highest acceptability followed by rose (16.89) and khus (16.0). Least was scored by chocolate flavored *ambil* (15.83).

4.1.4. Assessment of organoleptic properties through composite scoring for FOS added finger millet ambil with different flavors

The organoleptic scores of finger millet *ambil* with different flavors varied significantly with different flavors and colors.

The organoleptic attributes of the finger millet *ambil*s varied significantly with different flavors and colors. Rose and salt-cumin flavor both were equally (8.38) liked by the panel members. Least acceptability was for Khus (8.18) flavored *ambil*. The acceptability of taste and flavor varied significantly ranging from 23.22 to 25.15. Maximum was found for salt-cumin (25.15) flavored finger millet *ambil*, followed by rose (23.69), chocolate (23.24) and khus (23.22). Mouthfeel of the *ambil*s were also significantly different. Maximum score was obtained by salt-cumin (8.17) *ambil*. Slight reduction in the scores was reported from chocolate (7.71), rose (7.56) and khus (7.49) flavors. None of the flavors of beverage had shown any significant after taste. The scores ranged from 7.78 to 8.31, with no significant difference amongst all the flavors.

Overall acceptability showed a significant difference ($p<0.000$) with salt-cumin (17.19) flavor having the highest acceptability, preceded by chocolate (15.99) and rose (15.82).

4.2. Phase II – Probiotic profiling of buttermilk and standardized *Ambil*s in terms of strains of *Lactobacilli* and *Bifidobacteria*

4.2.1. Specificity of Primers

Novel species-specific primers for identification of the target species of Lactobacillus and Bifidobacterium were designed from 16S rRNA, 16S-23S rRNA intergenic spacer region, and 23S rRNA sequences. The specificity of the species-specific primer pairs was evaluated by PCR with

genomic DNAs purified from the reference strains of *L. rhamnosus, L. acidophilus, L. reuteri, L. reuteri, L. casei, B. adolescentis, B. bifidum, B. longum and B. lactis.*

4.2.2. Isolation of Bifidobacterium and Lactobacillus strains isolated from buttermilk

The sequence was compared with existing sequences in the NCBI database using the Blast N programme.

The *Bifidobacterium* strains isolated from buttermilk were *Bifidobacterium pseudocatenulatum strain B1279, Bifidobacterium catenulatum strain DSM 16992, Bifidobacterium angulatum strain B677, Bifidobacterium kashiwanohense strain HM2-2, Bifidobacterium ruminantium strain Ru 687, Bifidobacterium merycicum strain JCM 8219, Bifidobacterium callitrichos strain AFB22-5, Bifidobacterium stercoris strain Eg.* The DNA sequence of sample that matches best with that of *Bifidobacterium pseudocatenulatum strain B1279.*

The *Lactobacillus* strains isolated from buttermilk were *Lactobacillus acidophilus strain JCM 1132, Lactobacillus acidophilus strain NBRC 13951, Lactobacillus gallinarum strain JCM 2011, Lactobacillus gasseri strain ATCC 33820, Lactobacillus plantarum strain, Lactobacillus reuteri strain, Lactobacillus paracasei strain ATCC 25599, Lactobacillus crispatus strain DSM 20584, Lactobacillus crispatus strain ATCC 3382.* The DNA sequence of sample-1 matches best with that of *Lactobacillus acidophilus strain JCM 1132*

4.2.3. Isolation of Bifidobacterium and Lactobacillus strains isolated from rice ambil

The sequence was compared with existing sequences in the NCBI database using the Blast N programme.

The *Bifidobacterium* strains isolated from rice *ambil* were *Bifidobacterium stercoris strain Eg1, Bifidobacterium faecale strain CU3-7, Bifidobacterium adolescentis strain ATCC 15703, Bifidobacterium ruminantium strain Ru 687, Bifidobacterium catenulatum strain DSM 16992,*

Bifidobacterium kashiwanohense strain HM2-2, Bifidobacterium pseudocatenulatum strain B1279, Bifidobacterium angulatum strain B67. The DNA sequence of sample for Bif species matches best with that of *Bifidobacterium faecale strain CU3-7.*

The *Lactobacillus* strains isolated from rice *ambil* were *Lactobacillus acidophilus strain JCM 1132, Lactobacillus acidophilus strain NBRC 13951, Lactobacillus acidophilus strain VPI 6032, Lactobacillus acidophilus strain BCRC10695, Lactobacillus kitasatonis strain JCM 1039, Lactobacillus gallinarum strain ATCC 33199, Lactobacillus gallinarum strain JCM 2011, Lactobacillus crispatus strain DSM 20584, Lactobacillus crispatus strain ATCC 33820.* The DNA sequence of sample-1 matches best with that of *Lactobacillus acidophilus strain JCM 1132*

4.2.4. Isolation of Bifidobacterium and Lactobacillus strains isolated from barley ambil

The sequence was compared with existing sequences in the NCBI database using the Blast N programme.

The *Bifidobacterium* strains isolated from barley *ambil* were *Bifidobacterium faecale strain CU3-7, Bifidobacterium stercoris strain Eg1, Bifidobacterium adolescentis strain ATCC 15703, Bifidobacterium ruminantium strain Ru 687, Bifidobacterium kashiwanohense strain HM2-2, Bifidobacterium pseudocatenulatum strain B1279, Bifidobacterium catenulatum strain DSM 16992, Bifidobacterium angulatum strain B677.* The DNA sequence of sample-1 matches best with that of *Bifidobacterium faecale strain CU3-7.*

The *Lactobacillus* strains isolated from barley *ambil* were *Lactobacillus acidophilus strain JCM 1132, Lactobacillus crispatus strain DSM 20584, Lactobacillus crispatus strain ATCC 33820, Lactobacillus acidophilus strain VPI 6032, Lactobacillus kitasatonis strain JCM 1039, Lactobacillus plantarum strain ATCC 14917, Lactobacillus reuteri strain, Lactobacillus*

paracasei strain ATCC 25599, Lactobacillus gasseri strain ATCC 33820. The DNA sequence of sample-1 matches best with that of *Lactobacillus acidophilus strain JCM 1132.*

4.2.5. Isolation of Bifidobacterium and Lactobacillus strains isolated from pearl millet ambil

The sequence was compared with existing sequences in the NCBI database using the Blast N programme.

The *Bifidobacterium* strains isolated from pearl millet *ambil* were *Bifidobacterium bifidum strain NBRC 100015, Bifidobacterium bifidum strain DSM 20456, Bifidobacterium bifidum strain KCTC 3202, Bifidobacterium bifidum strain KCTC 3202, Bifidobacterium thermacidophilum subsp. porcinum strain P3-14, Bifidobacterium thermophilum strain AS 1.2235, Bifidobacterium scardovii strain CCUG 13008 A, Bifidobacterium pseudocatenulatum strain B1279, Bifidobacterium lemurum.*

The *Lactobacillus* strains isolated from pearl millet *ambil* were *Lactobacillus acidophilus strain JCM 1132, Lactobacillus acidophilus strain NBRC 13951, Lactobacillus casei strain JCM 1134, Lactobacillus casei ssp. pseudoplantarum strain, Lactobacillus kitasatonis strain JCM 1039, Lactobacillus plantarum strain ATCC 14917, Lactobacillus reuteri strain JCM 1112, Lactobacillus paracasei strain ATCC 25599, Lactobacillus gasseri strain ATCC 33820.* The DNA sequence of sample-1 matches best with that of *Lactobacillus acidophilus strain JCM 1132.*

4.2.6. Isolation of Bifidobacterium and Lactobacillus strains isolated from finger millet ambil

The sequence was compared with existing sequences in the NCBI database using the Blast N programme.

The *Bifidobacterium* strains isolated from finger millet *ambil* were *Bifidobacterium animalis subsp. lactis strain YIT 4121, Bifidobacterium animalis strain JCM 1190, Bifidobacterium pseudolongum subsp. globosum strain RU 224, Bifidobacterium choerinum strain Su 806,*

Bifidobacterium pseudolongum strain PNC-2-9G, Bifidobacterium gallicum strain P6, Bifidobacterium cuniculi strain AS 1.2239, Bifidobacterium animalis subsp. animalis strain ATCC 25527, Bifidobacterium breve strain DSM 20213. The DNA sequence of sample-1 matches best with that of *Bifidobacterium animalis strain YIT 4121.*

The *Lactobacillus* strains isolated from finger millet *ambil* were *Lactobacillus acidophilus strain JCM 1132, Lactobacillus acidophilus strain NBRC 13951, Lactobacillus acidophilus strain VPI 6032, Lactobacillus acidophilus strain BCRC10695, Lactobacillus kitasatonis strain JCM 1039, Lactobacillus plantarum strain ATCC 14917, Lactobacillus crispatus strain DSM 20584, Lactobacillus crispatus strain ATCC 33820, Lactobacillus gasseri strain ATCC 33820.* The DNA sequence of sample-1 matches best with that of *Lactobacillus acidophilus strain JCM 1132.*

Conclusively, it can be confirmed from the data that *Lactobacillus* strains predominant commonly in buttermilk and all the *ambil*s above 95% indent was *Lactobacillus acidophilus. Lactobacillus plantarum* and *Lactobacillus gasseri* was present in buttermilk, pearl millet, barley and finger millet *ambil*s. The common strain found in buttermilk, finger millet and barley *ambil*s were *Lactobacillus reuteri* and *Lactobacillus paracasei. Lactobacillus crispatus* was a strain that was found in buttermilk and all *ambil*s except pearl millet *ambil*.

Individual strains that were not identified in buttermilk but developed during the process of fermentation were *Lactobacillus kitasatonis* in all the four *ambil*s *and Lactobacillus casei* in pearl millet *ambil*.

Bifidobacterium strains predominant in rice, pearl millet and barley ambils with 99% indent was Bifidobacterium pseudocatenulatum, but was not identified in ragi *ambil*. *Bifidobacterium catenulatum, Bifidobacterium stercoris, Bifidobacterium adolescentis, Bifidobacterium ruminantium, Bifidobacterium kashiwanohense* and *Bifidobacterium angulatum* were commonly found in both rice and barley *ambil* as well as in buttermilk. It can also be observed from the data

that there were a few strains which were not identified in buttermilk but after fermentation developed in *ambil*s, these were *Bifidobacterium bifidum, Bifidobacterium thermacidophilum and Bifidobacterium scardovii* in pearl millet *ambil* and *Bifidobacterium animalis, Bifidobacterium pseudolongum, Bifidobacterium choerinum and Bifidobacterium gallicum* in finger millet *ambil*.

4.3. Phase III – Determination of nutritional profile and storage stability of fresh and packaged *Ambils* in terms of its physicochemical and microbial qualities

4.3.1. Physicochemical and microbial analysis - buttermilk and all four ambils

Moisture

The percent moisture in buttermilk was 95.85 and in rice, pearl millet, barley and finger millet *ambil*s were 84.87, 86.80, 87.17, 84.70 respectively. It can be observed that the percent moisture in barley *ambil* was higher than other *ambil*s followed by pearl millet *ambil*.

Acidity

Percent acidity of buttermilk was 0.50 and in rice, pearl millet, barley and finger millet *ambil*s were 0.51, 0.54, 0.56 and 0.51 respectively. The percent acidity of barley *ambil* was higher than other *ambil*s followed by pearl millet *ambil*.

pH

The average pH of buttermilk was 4.2 and in rice, pearl millet, barley and finger millet *ambil*s were 4.56, 4.58, 4.68 and 4.04 respectively. It can be observed that the pH of barley *ambil* was higher than other *ambil*s followed by pearl millet and rice *ambil*.

Carbohydrates

The carbohydrate content in 100ml buttermilk is 0.5 gm *(Gopalan, 2012)*. The carbohydrate content in rice, pearl millet, barley and finger millet *ambil*s were calculated to be 6.4, 5.59, 5.75 and 5.95 gms per 100ml respectively. The carbohydrate content in rice *ambil* was higher than other *ambil*s followed by finger millet *ambil*.

Protein

The protein content in buttermilk is 0.8 gms per 100ml *(Gopalan, 2012)*. The protein content in rice, pearl millet, barley and finger millet *ambil*s were calculated to be 1, 1.22, 1.22, 0.88 gms per 100ml respectively. It can be observed that the protein in barley and pearl millet *ambil* was higher than other *ambil*s followed by rice *ambil*.

Crude fiber

The average fibre in rice, pearl millet, barley and finger millet *ambil*s were calculated to be 0.01, 0.1, 0.32 and 0.29 gms per 100ml respectively, depicting that the fibre content in barley *ambil* was higher than other *ambil*s.

Total fat

The fat content of buttermilk is 1.1 gms per 100ml *(Gopalan, 2012)*. Total fat content in rice, pearl millet, barley and finger millet *ambil*s were calculated to be 0.45, 0.45, 0.52 and 0.9 gms per 100ml respectively

Calories

The total calorie content in buttermilk is 15 Kcal in 100ml *(Gopalan, 2012)*. Total calorie content in rice, pearl millet, barley and finger millet *ambil*s were calculated to be 33.3, 34.5, 32.5, and 31.9 Kcal per 100ml respectively. It can be observed that the total calorie content in pearl millet *ambil* was higher than other *ambil*s followed by rice *ambil*.

Total ash

Total ash content of buttermilk in percentage was 0.18 and in rice, pearl millet, barley and finger millet *ambil*s were 0.67, 0.73, 0.60 and 0.63 respectively. The percent ash content of pearl millet *ambil* was higher than other *ambil*s followed by rice *ambil*.

Vitamins

The folic acid content was found to be 2.07, 8.43, 2.54 and 3.10 mcg in rice, pearl millet, barley and finger millet *ambil*s respectively. Vitamin B content in rice *ambil* was found to be 0.01, 0.01, 0.09 for vitamin B1, B2 and B3 respectively. Vitamin B1, B2 and B3 content in pearl millet *ambil* was found to be 0.05, 0.05 and 0.03 respectively. Vitamin B1, B2 and B3 content in barley *ambil* was found to be 0.08, 0.04 and 0.91 respectively. Vitamin B content in finger millet *ambil* was found to be 0.07, 0.04, 0.06 for vitamin B1, B2 and B3 respectively. Vitamin B12 was found to be negligible in all the *ambil*s.

Minerals

Calcium content of buttermilk is 30 mg per 100ml *(Gopalan, 2012)*, and in rice, pearl millet, barley and finger millet *ambil* were calculated to be 12.2, 14.7, 13.4 and 38.9 mg per 100ml respectively. Highest being in pearl millet *ambil*. Sodium content of buttermilk was found to be 105.25 mg per 100ml *(Gopalan, 2012)*, and in rice, pearl millet, barley and finger millet *ambil* were calculated as

327, 328, 329 and 328 mg per 100ml respectively. Potassium content of buttermilk was found to be 151.18 mg per 100ml *(Gopalan, 2012),* and in rice, pearl millet, barley and finger millet *ambil* was calculated to be 60.7, 81.8, 123.8 and 62.2 mg per 100ml respectively. Highest being in barley *ambil*. Phosphorus content of buttermilk was found to be 30 mg per 100ml *(Gopalan, 2012),* and in rice, pearl millet, barley and finger millet *ambil* was calculated to be 24.2, 35, 28.6 and 34 mg per 100ml respectively. Highest to be seen in pearl millet *ambil* followed by finger millet *ambil.*

Viscosity

Viscosity of all the *ambil*s was determined. It was found to be 23.3, 21.5, 22.9 and 19.5 (mPa.s @20°) for rice, pearl millet, barley and finger millet *ambil*s respectively.

Peroxide value

Peroxide value of all the *ambil*s was determined. It was found to be less than 10 meq/Kg for rice, barley and finger millet *ambil*s. PV for pearl millet *ambil*s was found in the range of 10-20 meq/Kg.

Microbial analysis

Total Plate Count on day 0 for rice *ambil* was found to be 4.48 log 10 cfu g^{-1}, for pearl millet it was 4.42 log 10 cfu g^{-1}, for barley it was 4.61 log 10 cfu g^{-1} and for finger millet it was found to be 5.46 log 10 cfu g^{-1}. Depicting highest microbial growth in finger millet *ambil* followed by barley and pearl millet. Yeast and mold count for the *ambil*s were found to be 2.63 log 10 cfu g^{-1}, 2.66 log 10 cfu g^{-1}, 2.54 log 10 cfu g^{-1} and 2.72 log 10 cfu g^{-1} for rice, pearl millet, barley and finger millet *ambil*s respectively. Coliforms were absent in all the samples. Absence of coliform could

be due to high heat treatment given to the buttermilk while preparation and subsequent hygienic handling of the product.

4.3.2. Packaging of rice ambil and its nutritional evaluation

Rice *ambil* which was selected as best amongst all the *ambil*s based on organoleptic evaluation by trained panel members was packaged by Amul fed mother dairy, Ahmedabad. Tetra packaged *ambil* was subjected to physicochemical and microbial evaluation.

Nutritional profile of packaged rice ambil

The proximate composition of the packaged rice *ambil* was analysed. Total calorie content of the tetra pack was found to be 41.2 Kcal per 100ml. Protein and carbohydrate content was found to be 1.2gms and 8.4gms respectively. Total fat content was found to be 0.4gm per 100ml, saturated fat and PUFA was 0.27 and 0.11 gms respectively. Moisture and total ash was determined as 83.6% and 0.9% respectively. Acidity and pH was found to be 0.48% and 4.6 respectively.

Vitamin and mineral profile of the packaged rice *ambil* was analysed. Vitamin A and Vitamin B1 was found to be 48IU and 0.18 mcg respectively. Other vitamins were in negligible traces. Magnesium, calcium and phosphorus content was determined and was found to be 49mg, 84.6mg and 30.4mg respectively. Iron content was determined as 0.25 mg. Sodium and potassium content was found to be 238.1 mg and 33.4 mg respectively.

4.3.3. Storage stability of fresh ambils

All the freshly prepared *ambil*s were stored at refrigeration temperature of 4°C ±1 in glass bottles with a tight fitting lid. The storage stability was studied for a period of 5 days, or until it becomes unacceptable to beverage. Organoleptic characteristics and physical and microbial parameters

were studied including pH, acidity, viscosity, peroxide value, rancidity, whey separation, and microbial parameters including TPC, coliform, yeast and mold.

(i) Storage stability of rice ambil

Organoleptic evaluation

Freshly prepared rice *ambil* was stored at refrigeration temperature at 4°C ±1, there was a slightly significant ($p<0.05$) difference in color and appearance during the storage period of 5 days, starting from 8.9 from day one to 8.5 on day fifth. Consistency changed significantly ($p<0.000$) during the storage (8.8), which gradually decreased to 8 on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (8.6) to the end of five days (7) due to increase in acidity. A highly significant (0.000) change was observed in taste and flavor from 8.7 to 6. The scores for mouthfeel of rice *ambil* significantly ($p<0.000$) reduced from 8.8 to 6 at the end of day five. Similarly, after taste increased during the storage period, thus reducing the scores from 8.4 to 5.9. Overall acceptability scores showed a significant ($p<0.000$) decrease from 8.7 to 5.9, and was not much appreciated at the end of day 5. Thus it can be concluded that the acceptability of fresh rice *ambil* stored at refrigeration temperature of 4°C ±1 was till day 3.

Physical parameters

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared *ambil* was found to be 4.62, which was significantly reduced to 4.56, 4.52, 4.44, 4.17 and 3.94. A significant increase in acidity was also observed over the storage period, ranging from 0.32 on day zero to 1.61 on day five of storage. The peroxide value of the product was found to be less than 10 meq/Kg for all five days, thus confirm no rancid taste development throughout the storage. Viscosity of rice *ambil* was found to be 23.3 (mPa.s @20°) at day 0, which than gradually recorded as 23.3, 23.1, 22.9, 22.4 and 22.1 (mPa.s @20°) on day 1, day 2, day 3, day 4 and day 5

respectively. Whey separation index of different *ambil*s was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It was found to be 1.38 for day 0 which gradually increased to 1.60, 1.92, 2.20, 2.68 and 2.98 on day 1, day 2, day 3, day 4 and day 5 respectively.

Microbial parameters

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day one was found to be 4.48 (log 10 cfu g^{-1}), which showed significant increase from day 1 (4.66 log 10 cfu g^{-1}) to day 5 (5.86 log 10 cfu g^{-1}) respectively. Yeast and mold count on day one was found to be 2.63 (log 10 cfu g^{-1}) on day one, which upon storage in glass bottles at 4°C ±1 showed a significant increase ($p<0.05$) to 2.78, 2.82, 2.91, 3.28 and 3.72 on day 1, day 2, day 3, day 4 and day 5 respectively. Coliforms are an indicator of hygienic practices employed during product manufacture. It was found that coliforms were absent throughout the storage period in all the samples. Absence of coliform could be due to high heat treatment given to the buttermilk while preparation and subsequent hygienic handling of the product.

(ii) Storage stability studies for barley ambil

Organoleptic evaluation

Freshly prepared barley *ambil* was stored at refrigeration temperature at 4°C ±1, there was a significant ($p<0.0000$) difference in color and appearance during the storage period of 5 days, starting from 7.2 from day one to 6.3 on day fifth. Consistency changed significantly ($p<0.000$) during the storage (7.1), which gradually decreased to 5.7 on day five. Aroma scores showed a

significant difference ($p<0.000$) from day one (7.2) to the end of five days (5.5). Due to the decrease in pH during the storage period a significant (0.000) change was observed in taste and flavor (7.2 to 5), mouthfeel (7.1 to 4.7). Similarly, after taste increased during the storage period, thus reducing the scores from 6.9 to 4.6. Overall acceptability scores showed a significant ($p<0.000$) decrease from 6.8 to 4.6, and was not much acceptable at the end of day 5. Thus it can be concluded that the acceptability of fresh barley *ambil* stored at refrigeration temperature of 4°C ±1 was till day 2.

Physical parameters

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared *ambil* was found to be 4.74, which was significantly reduced to 4.68, 4.64, 4.51, 4.16 and 3.32. A significant increase in acidity was also observed over the storage period, ranging from 0.65 on day one to 1.12 on day five of storage. The peroxide value of the product was found to be less than 10 meq/Kg for all five days, thus confirm no rancid taste development throughout the storage. Viscosity of rice *ambil* was found to be 22.9 (mPa.s @20°) at day 0, which than gradually recorded as 22.9, 22.9, 22.9, 22.6 and 22.5 (mPa.s @20°) on day 1, day 2, day 3, day 4 and day 5 respectively. Whey separation index of different *ambil*s was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It was found to be 1.18 for day 0 which gradually increased to 1.24, 1.52, 1.80, 1.98 and 2.48 on day 1, day 2, day 3, day 4 and day 5 respectively.

Microbial parameters

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day one was found to be 4.61 (log 10 cfu g^{-1}), which showed significant increase from day 1 (4.77 log 10 cfu g^{-1}) to day 5

(5.68 log 10 cfu g^{-1}) respectively. Yeast and mold count on day one was found to be 2.54 (log cfu) on day one, which upon storage in glass bottles at 4°C ±1 increase ($p<0.05$) to 2.66, 2.79, 2.86, 3.02 and 3.22 on day 1, day 2, day 3, day 4 and day 5 respectively.

(iii) Storage stability of pearl millet ambil

Organoleptic evaluation

Freshly prepared pearl millet *ambil* was stored at refrigeration temperature at 4°C ±1, there was a significant ($p<0.0000$) difference in color and appearance during the storage period of 5 days, starting from 7.9 from day one to 6.4 on day fifth. Consistency changed significantly ($p<0.000$) during the storage (7.6), which gradually decreased to 6.6 on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (7.6) to the end of five days (6.5) due to increase in acidity. Due to the increase in acidity a significant (0.000) change was observed in taste and flavor (8.7 to 6), mouthfeel (7.2 to 4.2). Similarly, after taste increased during the storage period, thus reducing the scores from 6.8 to 4.1. Overall acceptability scores showed a significant ($p<0.000$) decrease from 7.1 to 4.9, and was not much acceptable at the end of day 5. Thus it can be concluded that the acceptability of fresh pearl millet *ambil* stored at refrigeration temperature of 4°C ±1 was till day 2.

Physical parameters

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared *ambil* was found to be 4.58, which was significantly reduced to 4.50, 4.46, 4.42, 3.90 and 3.64. A significant increase in acidity was also observed over the storage period, ranging from 0.45 on day one to 1.24 on day five of storage. The peroxide value of the product was found to be less than 10 meq/Kg for all five days, thus confirm no rancid taste development throughout the storage.

Viscosity of rice *ambil* was found to be 21.5 (mPa.s @20°) at day 0, which than gradually recorded as 21.4, 21.9, 21.2, 21.1 and 22.1 (mPa.s @20°) on day 1, day 2, day 3, day 4 and day 5 respectively. Whey separation index of different *ambil*s was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It was found to be 1.64 for day 0 which gradually increased to 1.80, 1.98, 2.28, 2.46 and 2.68 on day 1, day 2, day 3, day 4 and day 5 respectively.

Microbial parameters

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day one was found to be 4.42 (log 10 cfu g^{-1}), which showed significant increase from day 1 (4.98 log 10 cfu g^{-1}) to day 5 (5.86 log 10 cfu g^{-1}) respectively. Yeast and mold count on day one was found to be 2.66 (log cfu) on day one, which upon storage in glass bottles at 4°C ±1 increase ($p<0.05$) to 2.74, 2.86, 2.98, 3.48 and 3.67 on day 1, day 2, day 3, day 4 and day 5 respectively.

(iv) Storage stability studies for finger millet ambil

Organoleptic evaluation

Freshly prepared rice *ambil* was stored at refrigeration temperature at 4°C ±1, there was a slightly significant ($p<0.05$) difference in color and appearance during the storage period of 5 days, starting from 7 from day one to 5.1 on day fifth. Consistency changed significantly ($p<0.000$) during the storage (6.9), which gradually decreased to 5.3 on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (6.8) to the end of five days (3.9) due to increase in acidity. A drastic change in taste and flavor was observed which was highly significant (0.000), the scores ranged from 6.6 to 2.8. The scores for mouthfeel of rice *ambil* significantly ($p<0.000$) reduced

from 6.6 to 3.4 at the end of day five. Similarly, after taste increased during the storage period, thus reducing the scores from 5.9 to 3.5 Overall acceptability scores showed a significant ($p<0.000$) decrease from 5.2 to 2.8, and was not appreciated and acceptable at the end of day 5. Thus it can be concluded that the acceptability of fresh finger millet *ambil* stored at refrigeration temperature of 4°C ±1 was till day 2.

Physical parameters

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared *ambil* was found to be 3.97, which was significantly reduced to 3.84, 3.82, 3.68, 3.60 and 3.56 on day 5. A significant increase in acidity was also observed over the storage period, ranging from 0.45 on day one to 1.47 on day five of storage. The peroxide value of the product was found to be less than 10 meq/Kg for all five days, thus confirm no rancid taste development throughout the storage. Viscosity of rice *ambil* was found to be 19.5 (mPa.s @20°) at day 0, which than gradually recorded as 19.5, 19.5, 19.4, 19.4 and 19.1 (mPa.s @20°) on day 1, day 2, day 3, day 4 and day 5 respectively. Whey separation index of different *ambil*s was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It was found to be 1.82 for day 0 which gradually increased to 2.10, 2.38, 2.56, 2.94 and 3.22 on day 1, day 2, day 3, day 4 and day 5 respectively.

Microbial parameters

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day one was found to be 5.46 (log 10 cfu g^{-1}), which showed significant increase from day 1 (5.96 log 10 cfu g^{-1}) to day 5 (6.14 log 10 cfu g^{-1}) respectively. Yeast and mold count on day one was found to be 2.72 (log cfu)

on day one, which upon storage in glass bottles at 4°C ±1 increased ($p<0.05$) to 2.98, 3.36, 3.68, 3.92 and 4.12 on day 1, day 2, day 3, day 4 and day 5 respectively.

4.3.4. Storage stability of packaged rice ambil

The packaged rice *ambil*, was stored at room temperature. The storage stability was studied for a period of 6 months including 0 day, 15th day, 30th day, 60th day, 3 months and 6 months. Organoleptic characteristics and physical and microbial parameters were studied including pH, acidity, viscosity, peroxide value, rancidity, TPC, coliform, yeast and mold.

Organoleptic evaluation

The storage stability was studied for a period of 6 months including 0 day, 15th day, 30th day, 60th day, 3 months and 6 months. It was found that there was no change in the organoleptic paarameters including color, consistency, taste, mouthfeel, absence of defects, after taste and overall acceptability. The packaged *ambil* was highly acceptable throughout the storage period, with good organoleptic scores. In packaged rice *ambil*, there was no significant difference in colo and appearance during the storage period of 6 months, with no significant difference ($p>0.005$) starting from 8.9 from day one to 8.8 after 6 months. Consistency also remained unaltered until 120 days (8.9), which gradually decreased to 8.6 but still the difference was not significant. Aroma scores showed a significant difference ($p<0.000$) from day one (9) to the end of 6 months (8.6). Taste and flavor remained unaltered throughout 6 months. The scores for mouthfeel of packaged *ambil* significantly ($p<0.05$) reduced by 5% throughout the period of 6 months. Overall acceptability scores decreased from 8.97 to 8.73, with a significant decrease ($p<0.01$), but still it was highly acceptable at the end of 6 months.

Physical parameters

pH of packaged rice *ambil* on day 0 was found to be 4.6 and remained constant throughout the study period of 6 months, hence no significant difference. Acidity on day 0 was found to be 0.36 which was maintained throughout. Similarly no significant difference was seen in the viscosity, peroxide value and whey separation as they remained constant to 21.8 mPa.s @20°, peroxide value <10 meq/kg and whey separation 1.12 (expressed as ml whey expelled/ 10 ml of *ambil*) throughout the study period of 6 months.

4.4. Phase IV - Determination of Consumer acceptability and market potential of the most acceptable *Ambil*

4.4.1. Consumer acceptability

*F*ive hundred and eighteen consumers were selected for this research, out of them 303 were female and 215 male with 35.3 % were less than 20 years of age, 56.7% were in the age range of 20- 59 yrs and remaining 7% were above 60 years of age. All the consumers were given packaged rice *ambil* to taste and were given a hedonic rating scale to score the product.

Out of all the age groups defined for the study, the packaged *ambil* was liked most by the elderlies above 60 years of age with an average liking of 8.52 out of 9 on the hedonic rating scale. The average liking by other age groups closely followed by the elderlies was 7.96, 7.74, 7.71 and 7.56 by 40-59 years, 20-39 years, 10-19 years and less than 10 years respectively.

In the age group of less than 10 years 84% of the consumers graded the product 7 and above on hedonic scale, in the age group of 10-19 years 88% of the consumers graded the product 7 and above on hedonic scale, in the age group of 20-39 years 92% of the consumers graded the product 7 and above, for the age group 40- 59 years 95% of the consumers graded the product 7 and above and lastly for the age group of >60 years 98% of the consumers graded the product 7 and above.

Data from the consumer acceptability showed a significant difference ($p<0.000$) amongst the likings of each age group, and the data depicted that likability of the age group increases with the age group.

4.4.2. Market potential

Four hundred and twenty nine consumers were selected to study the market potential of the product. Eighty eight percent consumers in the age group of 10-19 years, 89% percent consumers in the age group of 20-39 years, 86% percent consumers in the age group of 40-59 years said that the product is a great idea and sounds interesting. Ninety seven percent consumers in the age group of >60 years said that the product is a great idea.

Another parameter studied for the market potential was whether the product is needed commercially or not (options given “yes”, “no” and “may be”). All the consumers were in favor of the product that something like this is needed, and 58%, 50%, 50.4% and 63% agreed from the specific age groups of 10-19 years, 20-39 years, 40-59 years and >60 years respectively. Out of all the age groups 29% were not sure whether it is needed or not and thus selected “may be” as an option from the choices given.

Purchase intent was studied on a scale of “yes”, “no” and “may be”. Purchase intent results agreed with those for product acceptability. Ninety three percent consumers said they will be willing to purchase the beverage if commercially available in market, and also were willing to recommend it to others if available. Expected price range was also asked from the consumers for a 200ml package. Majority of the consumers (42%) suggested a price range of Rs.10-15 for the product, 33% suggested a price range of Rs. 15-20 for the product, remaining consumers were willing to pay above Rs. 20 for the product if available commercially.

Preferred flavors were also studied according to the consumers liking. Twenty six percent consumers preferred chocolate flavor, closely followed by salt cumin flavor with 20%, 17% consumers preferred the mango and rose flavors, remaining consumers voted for other flavors including saffron, cardamom and pineapple.

Preferred package size of the product was also studied according to the consumer preference, given options were 100ml, 150ml, 200ml, 250ml or >250 ml package size. Forty two percent consumers preferred a 200ml package size for the product, followed by 27% for 100ml, 22% for 150ml. Not many consumers were in favour of a package size larger than 250ml.

The buying behavior of the consumers for milk products and health beverages available in the market was also investigated. It was recognized that the consumer buying behavior was mainly influenced by the quality of the product, in which the most important attribute was product's taste, followed by nutritional value, price and brand.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Modern life style, exposure to several environmental and physiological stress in day-to-day life have resulted in manifold multiplication of health problems such as cardiovascular and neurodegenerative diseases, anorexia, diabetes, cancer, especially with ageing. Due to growing awareness over nutrition and general well being, modern consumers throughout the world are trying to take a proactive position concerning their health. In this direction, probiotics and probiotic based food supplementation have drawn interest of consumers. The consumption of probiotics exerts myriad of beneficial effects which is evidenced by rigorous scientific evaluation. The technological application of the fermented beverage aims to combine the potential health benefits of the good bacteria with their ability to grow in with cereals, resulting in a nutritionally healthy and desirable product for the consumers.

The following conclusions and recommendations can be drained from the study:

Phase 1 of the study concluded that, out of all the *ambil*s, rice *ambil* was accepted the best. Rose and salt-cumin flavours had shown the maximum acceptability. Color, consistency, taste and overall acceptability of ragi *ambil*s was least acceptable amongst all the *ambil*s. Thus, FOS can also be added to other beverages without affecting their organoleptic attributes and can form part of daily diets of consumers. FOS can also be recommended as therapeutic products for its use in improving the outcomes of various metabolic disorders. FOS will enhance its functional value of the product.

From *Phase II* of the study, concluded that all fresh *ambil*s contain probiotic strains of *Lactobacillis* and *Bifidobacterium*. The predominant probiotic strains identified above 98% indent were *Lactobacillus acidophilus (100% indentation), Lactobacillus gallinarum, Lactobacillus casei, Lactobacillus paracasei and Lactobacillus casei.* Predominant *Bifidobacterium* strains identified were *Bifidobacterium pseudocatenulatum, Bifidobacterium catenulatum* and *Bifidobacterium angulatum.*

Results also depicted that there were few important individual strains that were not identified in buttermilk but developed during the process of fermentation, these were *Lactobacillus kitasatonis, Lactobacillus casei, Bifidobacterium bifidum, Bifidobacterium thermacidophilum,, Bifidobacterium animalis, Bifidobacterium pseudolongum, and Bifidobacterium gallicum.*

Phase III, of the study concluded that, all the fresh *ambil*s as well as the packged rice *ambil* had adequate nutritional composition. Storage stability studies for fresh *ambil*s prove that rice *ambil* was liked the best by the panel members and had better storage stability than other three *ambil*s. Results depicted that the *ambil*s were acceptable till day 3 with a taste score of 7.3 for rice *ambil*, 5.4 for pearl millet, 6 for barley *ambil* and 4.2 for finger millet *ambil*. The taste and mouthfeel score decreased significantly (<0.000) after day 4. Best acceptability till day 3 was that of rice *ambil* over other *ambil*s.

Storage stability of packaged rice *ambil* was acceptable over the entire 6 months period and there was no significant difference in the taste and acceptability.

Results of phase *Phase IV* of the study revealed that, the packaged rice *ambil* had a good acceptability amongst all the age groups, and was liked best by the elderlies(>60 years), very closely followed by the age group of 40-59 years and 20-39 years. Data from the consumer acceptability also depicted that likability of the age group increases with the age with significant

difference ($p<0.01$). The beverage was also subjected to its market potential, data collected revealed that the consumers feel something like this health beverage is needed commercially and were willing to purchase this product if available in markets as well as were willing to recommend it to other people as well.

Thus it can be concluded from the study that, the product *ambil* with added FOS have high sensory qualities and acceptability amongst all the age groups. FOS can also be added other beverages without affecting their organoleptic attributes and can form part of daily diets of consumers. All fresh *ambil*s contain probiotic organisms and have adequate nutritional value. Storage studies documented that fresh rice *ambil* is stable and acceptable until day 3 of storage and pearl millet, barley and finger millet *ambil* have a storage value of 2 days. Packaged rice *ambil* was acceptable throughout the study period of 6 months, thus confirming that if commercially packaged it has a good storage life. Finally, it can be concluded that, *ambil* has good consumer acceptance and market potential. Since *ambil* has a good probiotic content and nutritional value it can also be recommended as therapeutic product for its use in improving the outcomes of various metabolic disorders.

CHAPTER 6

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