DEVELOPMENT AND QUALITY EVALUATION OF PROBIOTIC HONEY BEVERAGE

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DECLARATION

I, hereby declare that this thesis entitled **"Development and Quality Evaluation of Probiotic Honey Beverage"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Development and Quality evaluation of Probiotic Honey Beverage"** is a record of bonafide research work done independently by Ms. Aparna H. Nath (2013-16-103) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

%	Per cent
^{0} brix	Degree brix
°C	Degree Celsius
CD	Critical difference
cfu/ml	Colony forming units per millilitre
et al.	And other co workers
Fig.	Figure
g	Gram
g/100g	Gram per 100 gram
h.	Hours
i.e.	that is
Kcal/100g	Kilocalories per 100 gram
ml	Milli litre
mg	Milli gram
viz.	Namely
pH	Negative logaritham of hydro carbon ion

DEDICATED TO MY FAMILY

INTRODUCTION

1. Introduction

In the industrialized world, the concepts in nutrition have changed significantly during the last 60 years. Existing scientific data show that both nutritive and non-nutritive components in foods have the potential to modulate specific target functions in the body, which are relevant to well-being and health and/or reduction of some major chronic and degenerative diseases, such as cardiovascular diseases, obesity, gastrointestinal tract disorders and cancer.

The best way to reduce lifestyle diseases is by the inclusion of functional foods in our daily diet. Functional foods can be defined as "food products that provide specific health benefits beyond the traditional nutrients they contain or foods containing significant level of biologically active components that impart health benefits beyond basic nutrition (Harrison, 2002). The increasing demand in this functional food is a response to the consumer demand for health food options (Menrad, 2002)

Thus, the era of functional foods started emerging. The term "functional food" was first used in Japan, in the 1980s, for food products fortified with special constituents that possess advantageous physiological effects.

Today, functional foods constitute the fastest growing sector in the food industry. They largely represent healthier versions of mainstream foods and drinks, and thus allow consumers to eat and drink more nutritiously without radically changing their diet (Holzapfel *et al*., 2013). Functional foods are also known as designer foods, medicinal foods, neutraceuticals therapeutic foods, super foods, foodiceuticals and medifoods.

Probiotics have a long history of human use and are traditionally consumed in several parts of the world. The concept of ''probiotics'' has attracted much attention with the emergence of antibiotic resistant bacteria and natural ways of suppressing pathogens (Tharmaraj and Shah, 2004).

Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that are associated with the beneficial effects for humans and animals. The root of the word 'probiotic' originated from Greek word ***pro*** meaning promoting and ***biotic*** meaning life together means '***for promoting life'***.

The development of probiotics during the past decade has signaled an important advance in the food industry transferring towards the development of such foods (Ouwehand *et al.*, 2002; Saad *et al.*, 2012). Probiotics are safe for human consumption and no reports have found on any harmfulness or production of any specific toxins by these strains. (Wright and Axelsson, 2001) .

Microbes from many different genera are being used as probiotics. The most commonly used strains belong to the heterogeneous group of lactic acid bacteria (*Lactobacillus, Enterococcus*) and to the genus *Bifidobacterium* (Ouwehand *et al.*, 2002; Saad *et al.*, 2012). However, other microbes and even yeasts have been developed as potential probiotics during recent years (Ouwehand *et al.*, 2002).

With an increase in the consumer vegetarianism, there is high demand for the vegetarian probiotic products (Vasudha and Mishra, 2013). The development of new nondairy probiotic food products is very much challenging, as it has to meet the consumer's expectancy for healthy benefits. (Stanton *et al.*, 2001).

At present, probiotic bacteria are mainly incorporated into dairy products, such as cheese, yogurt, ice cream and other dairy desserts. Fermented dairy products are the most traditional source of probiotic strains of lactobacilli; however, probiotic lactobacilli have been added to cooked pork meat products, snacks, fruit juice, chocolate and chewing gum (Bernardeau *et al.*, 2006; Ouwehand *et al.*, 2002; Ranadheera *et al.*, 2010).

Limitations of dairy products, such as the presence of allergens and the requirement for cold storage facilities, as well as an increasing demand for new foods

and tastes have initiated a trend in non-dairy probiotic product development. Further, it is important to develop probiotic products with food and beverages that are part of day-to-day normal diet to maintain minimum therapeutic level easily (Ranadheera *et al.*, 2010).

Keeping in view of the above aspects, the present study was taken up to develop a consumer acceptable probiotic honey beverage and to evaluate its chemical, nutritional, organoleptic and shelf life quality. This will enable to gain additional income to the honey growers.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The literature of present study entitled "Development and quality evaluation of probiotic honey beverage" is presented below.

2.1 Significance of Functional foods

2.2 Probiotics- Definition, characteristics/properties and mode of action.

2.3 Nutritional and therapeutic role of Probiotics.

2.4 Application of Probiotics in Foods and Beverages

2.5 Safety of Probiotics

2.6 Labeling and regulations governing probiotics.

2.1 Significance of functional foods

Development of foods that promote health and well-being is one of the key research priorities of food industry (Yoon *et al*., 2004). According to Jones and Jew (2007) functional food development has enjoyed heightened interest by commercial, academic and governmental sectors over the past decade. The trend has favoured consumption of functional foods that enriched with physiologically active components such as prebiotics, probiotics, vitamins, minerals, dietary fiber, plant sterol and other functional ingredients (Betoret *et al*., 2011)

Functional foods are similar in appearance to conventional foods; the former being consumed as part of the normal diet. In contrast to conventional foods, functional foods, have demonstrated physiological benefits which can be the risk of chronic disease beyond basic nutritional functions, including maintenance of gut health. FAO (2002) reported that when food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food".

Versatility in consumption with added health factors in addition to nutrition and flavor are characteristics of a functional food. In addition to exorbitantly high-priced health care and medicines, the desire for better quality of life (Vasiljevic, 2008) the link between diet and health is growing stronger day by day. Healing an illness through particular food consumption to restore natural defense with fewer side effects than medicine is always appealing to all age groups (Reid *et al*., 2001).

2.2 Probiotics - Definition, Characteristics and mode of action.

According to FAO (2009) the main functional groups for food processing are beneficial microorganisms (fermentation and probiotics). Microbial food cultures include bacterial food cultures, fungi and yeasts. These microorganisms determine the characteristics of the fermented food, e.g., acidity, flavour and texture, as well as health benefits that go beyond simple nutrition (Vogel *et al*., 2011). The market of functional food is dominated by gut health products, in particular probiotics. The global market for probiotic ingredients, supplements, and foods is expected to reach $19.6 billion in 2013, with more than 500 probiotic products introduced in the past decade alone (Ghishan and Kiela, 2011; Siró *et al*., 2008).

2.2.1 Definition of Probiotics

Foods containing probiotic microorganisms come within the category of functional foods which have a positive effect on health (Stanton *et al*., 2001).

DeVrese and Schrezenmeir (2008) stated that Probiotics are microorganisms, basically bacteria that when ingested would confer health benefit beyond the basic nutrition. Marteau *et al*. (2001) defined probiotics as microbial cell preparations or compacts of microbial cells that have a beneficial effect on the health and well being. According to Williams (2010) Probiotics are live non-pathogenic bacteria with the potential of colony formation in the gastrointestinal tract of humans. Sanders and Macro (2010) were of the opinion that to convey probiotics to human gut, a carrier system is needed which protect them against gastric acid.

The applicability of probiotics in food products depend on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH, mechanical stress, salt content and other essential ingredients (Mattila - Sandholm *et al*., 2002)

2.2.2 Probiotic Organisms

Lactobacilli are often considered to be commensal or beneficial participants in human microbial ecology and considerable research is being carried out on the effects for the use of lactobacilli as additives in both human and animal diets (Hummel *et al*., 2007). These bacteria have been used widely in dairy and non dairy products (Holzapfel and Schillinger, 2002)

Ng *et al*. (2009) reported that *Lactobacillus acidophilus* is a well known and well studied probiotic microorganism. *Lactobacilli* are highly competitive largely due to their applications in the production of fermented food. They can also produce antimicrobial substances including bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria (Rattanachaikunsopon and Phumkhachorn, 2010).

2.2.3 Characteristics/ Properties of the probiotics.

Certain important physiological characteristics of probiotics are resistance to stomach acid, pancreatic secretions such as bile and digestive enzymes in order to survive in high numbers in the small intestine. Fuller (2001) stated that properties of a good probiotics are safety, performance and technological properties.

Ross *et al*. (2005) viewed that initial assessment of basic probiotic characteristics may give an insight into their performance during harsh processing conditions. In order to qualify as a potential probiotic species, the microbes should possess initial screening characters for: acid resistance, bile salt tolerance, antibiotic resistance, cholesterol assimilation, antimicrobial and antimutagenic activity, adherence to epithelial cell wall and immuno-modulation and stimulation etc (Kailasapathy and Chin, 2000; Ledeboer *et al*., 2006; Prado *et al*., 2008).

Taumola *et al.* (2001) reported that good probiotics should be capable of exerting a beneficial effect on the host, non-pathogenic and non-toxic, should present in large numbers in viable cells, should be capable of surviving and metabolizing in the gut environment.

According to Wright and Axelsson (2001) characteristics of probiotics will determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period

Arora *et al*. (2012) opined that probiotic preparations should be GRAS- Generally Safe Regarded as Safe, resistant to bile, hydrocholoric acid and pancreatic juice and have anti carcinogenic activity, stimulate immune system, reduce intestinal permeability, produce lactic acid, able to survive both acidic condition of stomach and alkaline condition of the duodenum.

2.2.4 Mode of Action of probiotics

According to De Vrese & Marteau (2007), mechanism and efficiency of probiotic effect depend mainly on the interactions between probiotic microorganisms and microbiota of the host or with immuno competent cell of the intestinal mucous. Some microorganisms modulate glycosylation of the intestinal mucus and reduce the production of antimicrobials by the mucosa, revealing proposed mechanisms where by intestinal microbes influence the gut micro-ecology and shape the immune system. (Shinde, 2012)

According to Oelschlaeger (2010) three modes of actions of probiotics are i) They may be able to modulate the host's defences including the immate as well as the acquired immune system. ii) Probiotics can have direct effect on other microorganisms, and or pathogenic ones.iii) Probiotic effects may be based on action affecting microbial products like toxins and host products like bile salts and food ingredient.

Sanders (2008) reported probiotics can modify the surrounding environment by modulating the pH or the oxidation reduction potential, which may compose the ability of pathogens to become established. They may provide beneficial effects by stimulating non-specific immunity and modulating the harmful and cellular immune response.

Some of the probiotics particularly lactic acid bacteria inhibit the growth of the pathogens by creating an acidic environment through the production of organic acid (Ogava *et al*., 2001)

Probiotics have various mechanisms of action although the exact manner in which they exert their effects is still not fully elucidated. These range from bacteriocin and short chain fatty acid production, and lowering of gut pH and nutrient. (Guarner and Malagelada,2003). Competition to stimulation of mucosal barrier function and immune modulation has been the subject of numerous studies. There is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing the responses and attenuating Th_2 response (Guarner,2003).

Probiotic bacteria cultures encourage growth of beneficial micro organism and crowd out potentially harmful bacteria there by reinforcing the body's natural defence mechanism. (Saarela *et al*., 2000)

According to Yao *et al*. (2002) Probiotic effect is accredited to the production of metabolic by product such as acid, hydrogen peroxide, bacteriocin etc. Diaz *et al*. (2008) found that probiotics enhance intestinal epithelial barrier functions by increasing the production of mucin, preventing injury of the epithelium from pathogens and reducing cell permeability. They may also enhance the mucosal barrier function by inducing expression of antimicrobial peptides like defensins.

The action mode depends on the metabolic properties, the kind of surface molecules expressed and the components to be secreted (Santosa *et al*., 2006).

2.2.5 Shelf life Qualities of Probiotic

Viability of probiotic is reduced as a result of their exposure to high temperature, oxygen, low pH and light (Chen and Yao, 2002). The viability of probiotics must be maintained during storage and processing in order to exert their beneficial effects on the cultured species. Lactobacilli strain showed good cellular stability maintaining constant concentration throughout the storage period regardless of final pH (Donkor *et al*., 2008). Methods for improving probiotics viability are gene modification, immobilization, two-step fermentation, use of oxygen, impermeable containers and microencapsulation (Ozer *et al*., 2010). Microencapsulation technique is widely exploited to improve the shelf life and to retain health properties of probiotics (Semyonov *et al*., 2010).

2.3 Nutritional and therapeutic Role of Probiotics

2.3.1 Nutritional Significance of probiotics

Ingestion of probiotics is associated with improved production of riboflavin; niacin, thiamine, vitamin B_6, vitamin B_{12}, and folic acid .Probiotics play a role in increasing bioavailability of calcium, iron, manganese, copper phosphorous and increase the digestibility of protein and fat in yogurt. Microbial action in the gut, specifically by beneficial cultures, has been shown to enhance the bioavailability and digestibility of certain nutrients and also the organic acids such as acetate and lactate produced during fermentation by LAB (Parvez *et al*., 2006).

2.3.2 Therapeutic effects of probiotic foods.

Kalliomaki *et al*. (2001) reported that probiotics can play a role in immunological, digestive functions and have effect in alleviating infectious diseases in children as well as in adults

Probiotics are one of the prime gut microflora inherited by infants from their mother's vagina as well as through breast feeding. It has been demonstrated that these microbiota protect the gut from enteric pathogens (Wang *et al*., 2010). Shah (2007) opined that a number of health benefits have been claimed for probiotic bacteria and are also being recommended as a preventive approach to maintain the balance of intestinal microflora. Their beneficial effects on humans include i) stabilization of intestinal microflora (excluding colonization of entero-pathogenic bacteria by adhesion to the intestinal wall and competition for nutrients (Denev,2006), ii) reduction of lactose intolerance (De Vrese *et al*., 2001) iii) prevention of antibiotic-induced diarrhoea (Pochapin, 2000), iv) prevention of colon cancer (Wollowski *et al*., 2001), v) stimulation of the immune system (Isolauri *et al*.2001) etc.

2.3.2.1 Lactose Intolerance.

Tuoky *et al*.(2003) reported that probiotic bacteria contain high level of lactase which is released within the intestinal lumen and help indigestion. Fermented milk contains probiotics that survive the passage through the stomach, and finally deposited in the small intestine to support lactose hydrolysis by its own enzymes (De Vrese and Schrezenmeir ,2008). The best way to reduce lifestyle diseases is by the inclusion of functional foods in our daily diet. Functional foods can be defined as "food products that provide specific health benefits beyond the traditional nutrients they contain or foods containing significant level of biologically active components that impart health benefits beyond basic nutrition (Harrison, 2002). The increasing demand in this functional food is a response to the consumer demand for health food options (Menrad, 2002)

2.3.2.2 Diarrheal diseases

Probiotics can prevent or ameliorate diarrhea through their effects on the immune system. Vasiljevic (2008) reported that fermented milk products also

reduce the duration of symptoms of antibiotic associated diarrhea caused by clostridium *difficile* and rotavirus diarrhea. Several probiotic strains, especially *Lactobacillus rhamnosus* have been shown to prevent or alleviate infantile diarrhea, caused mainly by rotavirus. It is also well-established that some probiotic strains can prevent and shorten antibiotic-associated disorders. (Fuller *et al*., 2008)

2.3.2.3 Inflammatory Bowel Disease (IBD) & Irritable Bowel Syndrome

Inflammatory bowel disease comprises a spectrum of disorders characterized by inflammation, ulceration and abnormal narrowing of the gastrointestinal tract resulting in abdominal pain, diarrhea and gastrointestinal bleeding (Vasiljevic, 2008). Probiotic administration in IBD bring about relief either through regulation of the inflammatory response, enhancing barrier function to prevent the invasion of tight junctions or modulation of gut microbata composition and active symptoms and prevent remission (Santosa *et al*., 2006).

2.3.2.3.1 Pouchitis

Pouchitis is defined as acute or chronic inflammation of the ileal reservoir created after colectomy and ileal pouch-anal anastomosis.

Probiotics prevent initial attack of pouchitis and prevent further relapse of pouchitis after induction of remission with antibiotics. Probiotics can be recommended to patients with pouchitis of mild activity. (Guarner, 2008)

2.3.2.3.2 Ulcerative Colitis

The several probiotic compounds have shown promise in the therapy of ulcerative colitis. Bifidobacteria fermented milk has been found to decrease the rate of relapse. In mild to moderate ulcerative colitis, Saccharomyces *boulardii* given for 4 weeks induced remission in 17 to 24 patients.(Guarner,2008)

2.3.2.4 Helicobacter Pylori.

Lactobacilli and *bifidobacteria*l strains, as well as Bacillus *clausii* reduce the side effect of antibiotic therapies and improve patient compliance (Arora *et al*., 2012). Supplementation of anti-H. pyroli antibiotic regimens with probiotics effective in increasing eradication rates . Probiotics are helpful as adjuvant therapy with antibiotics in the eradication of H.pylori infections(Guarner, 2008)**.**

2.3.2.5 Hepatic Diseases

Hepatic Encephalopathy is a liver disease its effects are life threatening. The exact pathogenesis of HE still remains unknown. The probiotics strep.*thermophilus*,Bifidobacteria, L.*acidophilus*, L.*plantarum* L. *casei*, L.*delbruekkii bulgaricus* containing therapeutic effect have multiple mechanisms of action (Guarner,2008).

2.3.2.6 Constipation

Constipation, a disorder of motor activity of the large bowel Characterized by bowel movements that are less frequent than normal, pain during defecation abnormal swelling and incomplete emptying of colon contents can be relieved by probiotic use.

Guarner (2008) viewed that a majority of the clinical trials reviewed showed that lactic acid bacteria alleviate abdominal pain and discomfort. Both single and multi centre studies have shown that lactic acid bacteria may improve abdominal bloating and distension.

2.3.2.7 Immune function

The immune system is extremely complex involving both cell-based and antibody based responses. Exposure to foreign antigens elicits a complex cascade

of responses from the human body including launching protective reactions against food antigens and colonizing micro flora. The immune response may further be enhanced when one or more probiotics are consumed together and work synergistically (Guarner,2008) .

Sanders (2007) reported that probiotic cultures enhance levels of certain immune reactive cells and also provide an additional tool to help our body to protect itself.

Probiotics shown to have immunomodulatory properties through the inhibition of bacterial translocation, stimulation of phagocytes, macrophages and natural killer cells,increased proliferation in organs of the immune system and increased levels of cytokines. (De Vrese, 2008)

Probiotic bacteria are able to enhance both specific and non-specific immune response by activating macrophages increasing levels of cytokine, increasing level of immunoglobulin's especially IgA .

2.3.2.7 Cancer

In general cancer is caused by mutation or activation of abnormal genes that control cell growth and division. Probiotics were shown to posess antimutagenic and anticarcinogenic activity against well-known mutagens and pro mutagens although the mechanisms are still unknown. They can decrease levels of cellular enzymes responsible for the activation of pro carcinogens (Guarner,2003). Alternatively microbes can be involved in the metabolism of substance or into the prevention of their binding to the cell surface. (Rafter, 2003).

In the recent years, probiotics are also established to have "anti-cancer" properties, by detoxifying ingested carcinogens and altering metabolic activities of cancer-carrier bacteria (Ray, 2004; Parvez *et al*.,2006).

Sanders, (2008) reported that Probiotic cultures decrease the exposure chemical carcinogens by detoxifying ingested agents, altering the environment of the intestine and thereby decreasing population or metabolic activities of bacteria, producing metabolic products, producing inhibit growth of tumor cells, stimulating immune system to defend against cancer proliferation..

According to Sanders(2007) probiotic bacteria could counteract mutagenic and geotaxis effects in the colon and other organ sites. Probiotics play an important role in the prevention of cancer by detoxifying ingested carcinogens, altering the environment of the intestine and thereby decreasing population to inhibit the growth of tumor cells, stimulating immune system better defend against cancer cell proliferation.

Certain members of *Lactobacillus and Bifidobacterium* spp.decrease the level of carcinogenic enzymes produced by colonic flora through normalization of intestinal permeability and micro flora balance as well as production of antimutagenic organic acids and enhancement of hosts immune system .

2.3.2.8 Cholesterol

Although human synthesise cholesterol to maintain minimum levels for biological functioning, diet also known to play a role in serum cholesterol levels (Sanders, 2008).Several mechanisms have been hypothesized, which include enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics, assimilation of cholesterol by probiotics, co-precipitation of cholesterol with deconjugated bile, incorporation of cholesterol into the cellular membranes of probiotics during growth and conversion of cholesterol into coprostanol (Liong and Ooi,2010).The use of probiotics to reduce the risk of hypercholestremia seems to be very effective specially if consumed as a part of normal daily nutrition. *L.acidophilus* have high rate of cholesterol lowering effect (Vasiljevic, 2008).

2.3.2.9 Hypertension

Dietary recommendations accompany more aggressive strategies to control hypertension, and food products derived from probiotic bacteria could possibly contribute to blood pressure control (De Vrese,2008). Sanders (2007) found that consumption of lactobacilli or products made from them may reduce blood pressure in mildly hypertensive people.

2.3.2.10 Allergy

Hereditory allergic conditions are increasing importance in developing countries such as eczema, asthma, atopic dermatitis and rhinitis can be treated with probiotics (Kalliomaki *et al* ., 2001).

The prevalence of allergic diseases has increased over the last 35-40 years particularly in western societies. Probiotic bacteria are important in regulating inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy. Probiotics are also helpful in alleviating some of the symptoms of food allergies such as those associated with milk proteins (Sanders, 2008).

Exposure to bacteria in early life may exhibit a protective role in allergy and probiotics may provide safe alternative microbial stimulation needed for the developing immune system in infants (Guarner, 2003)

2.3.2.11 Infectious Diseases

Parassol *et al*. (2005) reported that Probiotics compete with pathogens for adhesion sites, strengthen the epithelial barrier by preservation of tight junction, protein expression between enterocytes and inhibition of epithelial cell apoptosis.

Probiotics are known to secrete anti microbial molecules. Currently, most beneficial effect of probiotics has been observed in studies on diarrhea, in particular rotavirus watery diarrhea (Rautava *et al*., 2006).

2.3.2.12 Kidney and Pancreas

Sanders, (2007) reported probiotics play a major role reducing kidney stones by improving GI tract oxalate levels and may decrease the oxalate absorption.

2.3.2.13 Post Surgery

Application of probiotics for surgical patients is not necessarily limited to skin and wounds .L.*plantarum* 299 gives with enteral fiber nutrition decreased the rate of post operative infections in liver transplant patient at very high risk of infections, organ rejection and death (Guarner, 2008)

2.4 Application of Probiotics in Foods and Beverages

One way in which foods can be modified to become functional by the addition of probiotics (FAO/WHO,2006).The presence of probiotics in commercial food products has been claimed for certain health benefits. This has led to industries focusing on different applications of probiotics in food products and creating a new generation of **'probiotic health' foods.** The range of food products containing probiotic strains is wide and still growing.

2.4.1 Dairy Based Probiotic Foods

Milk and its products is good vehicle of probiotic strains. Dairy products play important role in delivering probiotic bacteria to human. Probiotics can be found in a wide variety of commercial dairy products including sour and fresh milk, yogurt, cheese, etc.

Dairy products play important role in delivering probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability (Saarela *et al*., 2002)

Several factors need to be considered in dairy products such as viability of probiotics in dairy, the physical, chemical and organoleptic properties of final products, the probiotic health effect and the regulations and labeling issues. (Kruck, 2006)

2.4.1.1Drinkable fresh milk and Fermented Milk

Among probiotics carrier food products, dairy drinks were the first commercialized products that are still consumed in larger quantities than other probiotic beverages.

According to Ozer *et al*.(2010) functional dairy beverages can be grouped into two categories: fortified dairy beverages (including probiotics, prebiotics, fibers, polyphenols, peptides, sterol, stanols, minerals, vitamins and fish oil), and whey-based beverages.

Factors affecting the viability of probiotic cultures in fermented milks are acidity, pH, dissolved oxygen content, redox potential, hydrogen peroxide, starter microbes, potential presence of flavoring compounds and various additives (including preservatives) (Saarela *et al*., 2009)

2.4.1.1 Yogurt

Yogurt is one of the original sources of probiotics and continues to remain a popular probiotic product today. Yogurt is known for its nutritional value and health benefits. Yogurt is produced using a culture of *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* bacteria (Corrales *et al*., 2007). Although yogurt has been widely used as probiotics vehicle, most commercial yogurt products have low viable cells at the consumption time.

Viability of probiotics in yogurt depends on the availability of nutrients, growth promoters and inhibitors, concentration of solutes, inoculation level, incubation temperature, fermentation time and storage temperature, strains of probiotic bacteria, pH, buffering capacity of the media as well as the storage temperature (Talwalker and Kailasapathy, 2004)

An increased demand for non-dairy probiotic products comes from vegetarianism; milk cholesterol, milk allergy and others factors (Ray, 2004).

Encapsulation in plain alginate beads, chitosan coated alginate, alginate-starch, alginate- prebiotic, alginate-pectin, in whey protein-based matrix, or by adding prebiotics or cysteine into yogurt, could improve the viability and stability of probiotics in yogurt. (Venugopalan *et al*., 2010)

2.4.1.2 Cheese

Probiotics in cheese were found to survive through the simulated human gastrointestinal tract and significantly increase the numbers of probiotic cells in the gut (Ouwehand *et al.*, 2009). Cheeses have a number of advantages over yogurt and fermented milks because they have higher pH and buffering capacity, highly nutritious, high energy, more solid consistency, relatively higher fat content and longer shelf life.

Fresh cheese like cottage cheese has high recommended daily intake, limited shelf life with refrigerated storage temperature. Cheese serve as a food with a high potential to be applied as a carrier for probiotics (Vinderola *et al.*, 2000)

2.4.1.3 Other dairy products

Other dairy products including quark, chocolate mousse, frozen fermented dairy desserts, sour cream, and ice cream were good vehicles of probiotics. Ice creams are among the food products with high potential for use as probiotic vehicle. (Cruz *et al*., 2009)

Wilson *et al*. (2004) found that sour cream as feasible probiotic beverage. Probiotic cultures do not modify the sensory characteristics of the ice-creams and frozen desserts and hold good viability for probiotics during storage period.

2.4.2 Non dairy probiotic beverages

Most probiotic foods in the current market are refrigerated dairy-based products (Burgain *et al*., 2011) while preparations of non-dairy foods are attracting a broader range of consumers with different preferences.

Non-dairy probiotic foods offer an advantage for individuals with lactose intolerance and high cholesterol which is a major drawback of dairy-based probiotics (Prado *et al*., 2008). (Luckow and Delahunty, 2004; Yoon *et al*., 2006) were of the opinion that dairy allergens are absent in fruits and vegetables besides they contain dietary fibers, phytonutrients and antioxidants which makes them acceptable by almost all age groups of the population..

2.4.2.1 Cereal based

The development of new functional foods which combine the beneficial effects of cereals and health promoting bacteria is a challenging issue. Cereals are good substrates for the growth of probiotic strains, due to the presence of non-digestible components of the cereal matrix (Salovera ,2011).Some of the common probiotic foods we included in our daily diet are Idli,Ada, Dosa etc.

Mahewu (amahewu) is a sour beverage made from the maize porridge with predominant microorganism *Lactococcus lactis* subsp. *lactis*. (Blandino *et al*., 2003). Santos (2001) developed a probiotic beverage with the fermented cassava flour using mixed culture of *Lb. plantarum,* which were amylolytic strains of *Lb. casei Shirota* and *Lb. acidophilus*. Angelov *et al*. (2006) produced a symbiotic

functional drink from the oats by combining a probiotic starter culture and whole-grain oat substrate.

Togwa, a starch-saccharified traditional beverage consumed in Africa, is usually made from the maize flour and finger millet malt (Oi and Kitabatake, 2003) . Boza, a beverage consumed in Bulgaria, Albania, Turkey and Romania, is a colloidal suspension, from light to dark beige, sweet, slightly sharp to slightly sour, made from wheat, rye, millet, maize and other cereals mixed with sugar, or saccharine (Blandino *et al*., 2003).

Wacher *et al*. (2000) reported tha Pozol a refreshing beverage, consumed in the Southeastern Mexico, is made by cooking maize in an approximately 1% (w/v) lime solution.

2.4.2.2 Fruit based

Nowadays, there is increasing interest in the development of fruit-juice based probiotic products. The fruit juices contain beneficial nutrients that can be an ideal medium for probiotics (Tuorila *et al*., 2002).

Pomegranate juice was proved to be a suitable probiotic drink as results have shown desirable microbial growth and viability for *L.plantarum* and *L. delbruekii*. (Kourkoutas *et al*.,2011). Hardaliye is a lactic acid fermented beverage produced from the natural fermentation of the red grape, or grape juice with the addition of the crushed mustard seeds and benzoic acid.

Luckow and Delahunty (2004) evaluated the consumer'sacceptance for the appearance, aroma, texture and flavour of the probiotic fruit juices.

2.4.2.3 Vegetable based

Fermentation of vegetables has been known since ancient time. Fermented vegetables can offer a suitable media to deliver probiotics. Traditional methods of production might result in inactivation of the probiotic cultures and the

use of probiotics in fermented vegetables would require low temperature storage of the products (Champagne, 2009).Some of the vegetable based probiotics are vegetable based drinks like cabbage juice, carrot juice etc.

Yoon *et al*.(2006) reported that fermented cabbage juice supports the viability of probiotics and serves as a healthy beverage. .

2.5 Safety of Probiotics

Criteria for probiotics in foods is their safety for human consumption. Sanders, (2008) pointed out that characterization of a probiotic strain is based on the absence of resistance to clinical or veterinary antibiotics as well as the absence of virulence factors.

Factors important for assessing the safety of a probiotic include the method of administration, the level of exposure, the health status of the users, and the physiologic functions they are intended to perform. (Sanders and Macro, 2010)

The identification of bacterial strain is necessary not only for safety reasons, but also to prove their efficacy due to the fact that different strains of the same species may exert different effects on the host (FAO/WHO,2006).

The possible complications related to the use of probiotics could be the development of bacteriemia ,sepsis or endocarditis,the toxicity and the metabolic effects on the GI tract and the possibility of transfer antibiotic resistance to the GI flora (Vasiljevic,2008).

The food industry needs to carefully assess the safety and efficacy of all new species and strains of probiotics before incorporating them into food products.

2.6 Labeling and regulations governing probiotics

Appropriate labeling and heath claims are pre requisite for consumer to make a choice. In addition to the general labeling requirements under the food laws of each country necessary information should also be stated on the label (Hiller, 2001).

The indications on the label include various conditions such as immunomodulation, urinogenital infection and skin etc. It is the responsibility of manufacturers to give due consideration to the safety aspects (FAO/WHO, 2006)

From a scientific perspective, the suitable description of a product as reflected on the label should include (FA0/WHO, 2006) :-

- Genus and species identification, with nomenclature consistent with current scientifically recognized names.
- Strain designation.
- Viable count of each strain at the end of shelf life.
- Recommended storage conditions.
- Safety in the condition of recommended use.
- Recommended dose, based on induction of the physiological effect.

  An accurate description of the physiological effects, as far as allowable by law

FAO/WHO (2006) rightly pointed that the indications on the label include various conditions such as immunomodulation, urinogenital infection and skin etc. It is the responsibility of manufacturers to give due consideration to the safety aspects.

Depending on the intended use of a probiotic, whether as a food /food ingredient, a dietary supplement and or a drug, regulatory requirements differ greatly among countries (Reid *et al*., 2001).

The guidelines issued by FAO/WHO:-

- Proper identification to the level of strains of all probiotics in the product.
- Characterization of each strain for important to its safety and function.
- Validation of health benefits in human studies, including identification of the quantity of the micro organisms required to provide the benefit.
- Truthful and not misleading labeling of efficacy claims and content through the end of shelf life.

The application of probiotic cultures in non-dairy products and environments represents a great challenge and needs to be researched at the industrial level for commercial production of these healthy products (Mattila-Sandholm *et al*., 2002).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Probiotic foods gaining much popularity as an alternate approach for the healthcare management and proved are therapeutic indications from simple to complex diseases. Probiotics represent probably the archetypal functional food, and are defined as alive microbial supplement, which beneficially affect the host by improving its intestinal microbial balance. (Kalliomaki *et al*., 2001). In the present study, a non- dairy probiotic beverage was proposed to be developed with all the sensory qualities. Probiotic honey beverage would be an ideal choice as it serves as a good media for growth of microorganisms.

Methodology adopted for the conduct of the study entitled **"**Development and quality evaluation of Probiotic honey beverage" is described under the following:

3.1 Selection & procurement of ingredients.
3.2 Product formulation and standardisation
3.3 Quality assessment of the product.
3.4 Probiotication process of the beverage.
3.5 Quality analysis of the probiotic beverage
3.6 Shelf stability of the probiotic beverage.
3.7 Consumer acceptability of the probiotic beverage.
3.8 Cost and yield analysis.
3.9 Statistical analysis.

3.1 Selection and procurement of ingredients

3.1.1 Selection of ingredients

Honey, Aloevera pulp and Soya milk were the ingredients selected for the development of probiotic drink (Plate 1). The numerous health benefits of honey have made it an important element of traditional medicines such as ayurvedic treatment, while aloevera gel is used as one of the ingredient in yoghurts, beverage

Plate No: 1 . Ingredients used for the formulation of the beverage

HONEY

ALOEVERA LEAVES

SOYA MILK

and some desserts. Soya milk is a complete protein and has about the same protein as cow's milk, and a source of dietary fiber, vitamins and minerals.

3.1.1.1 Honey

The use of natural honey as food and medicine by man- kind has been in existence from time immemorial. Natural honey (NH) is a sweet, flavourful liquid food of high nutritional value (Ajibola *et al*., 2007) and immense health benefits (Bogdanov *et al*., 2008).

Honey is primarily made of water and carbohydrates. It also contains trace amounts of several minerals and vitamins. Honey also contains a blend of flavonoids and phenolic acids. These are antioxidants that eliminate potentially destructive free radicals in the human body (Sampath *et al*.,2010)

. The honey produced by Apis *cerana indica* is the most commonly reared bee species by the bee keepers and available in plenty in the market. In the present study honey from *Apis cerana indica* was used as main ingredient in the beverage.

3.1.1.2 Aloevera

Aloe vera is a hardy, perennial, tropical, drought-resistant, Succulent plant belonging to the Liliaceae family which, historically has been used for a variety of medicinal purposes. (Sampath *et al*., 2010).

The raw pulp of *A. vera* contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water (Eshun, K.2004.). The remaining 0.5 – 1% solid material consists of a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids (Boudreau and Beland, 2006). It has been hypothesized that this heterogenous composition of the *aloe vera* pulp may contribute to to the diverse pharmacological and therapeutic activities which have been observed for aloe gel products (Talmadge *et al*., 2004).

Sadiq *et al.* (2004) reported that *Aloe vera* juice is useful to treat gastric intestinal problems like indigestion, colitis and relief from digestive issues such as heartburn and irritable bowel syndrome. The anti-ulcer activities of *A. vera* has been attributed to several possible mechanisms including its anti-inflammatory properties, healing effects, mucus stimulatory effects and regulation of gastric secretions (Suvitayavat *et al*.,2004).

Above findings justify the selection of aloevera pulp in the formulation of the beverage which assumes to promote health.

3.1.1.3 Soymilk

The soybean plant (*Glycine max*) belongs to the *legumeacea* family. On average, dry soybean contains roughly 40% protein, 20% oil, 35% soluble (sucrose, raffinose stachyose, etc.) and insoluble (dietary fiber) carbohydrate and 5% ash. Fresh soybean has approximately 14% moisture (Liu, 2004) .

Soymilk and fermented soymilk products considered as a suitable economical substitutes for cow's milk and an ideal nutritional supplement for lactose intolerant population . (Pyo and Song,2009) Soyamilk also contains isoflavones, which can reduce the risk of most hormone-associated health disorders (Kurzer, 2000).

It is a good substitute for milk for such individuals because it contains no casein and lactose (Fiocchi *et al*., 2003). Because of low content of saturated fats,

high amounts of polyunsaturated fats, absence of cholesterol and presence of plant sterols, soymilk, unlike milk possesses anticholesterolemic and antiatherogenic properties (Sacks *et al*., 2006; Gardner *et al*., 2007).

Soymilk is a suitable food to be included in a person's diet who have lactose allergy. Champagne *et al.* (*2009*) had pointed out that soy is considered as a good substrate for functional foods, since fermentation by probiotics has the potential to reduce the levels of some carbohydrates and favor desirable changes in bacterial populations in the gastrointestinal tract.

3.1.2 Procurement of ingredients

Honey was procured from local honey growers in Thiruvananthapuram. Aloevera leaves were plucked fresh from the fields.. maintained for cultivation purposes. Soymilk packed in tetra packs was purchased as and when required observing date of manufacture. Care was taken to obtain fresh soya milk. as far as possible.

3.1.2.1 Preliminary processing of Aloevera Pulp

Aloevera leaves were washed thoroughly with running water to remove dirt and other extraneous matter on the leaves. Leaves were kept to drain out water in a colander. Leaves were kept at low temperature for 2- 3 hours to make the gel solidified. After that each leaf was carefully peeled off, starting from the both edges first and then the flat side of the leaves. Gel was thus separated from the leaves, washed once again and blended well in a blender to get sooth gel. The aloevera pulp and water required for formulation were transferred to the sterilized glass bottles in the laminar air flow chamber. The aloevera pulp was autoclaved and cooled and mixed with other ingredients to make it a consumer acceptable product.

3.2 Product formulation and Standardisation.

3.2.1 Optimization of ingredients for probiotic drink

First step in the formulation of the drink was to optimize the ingredients in an appropriate proportion. Since honey is the basic ingredient the, proportion of honey in the drink should be at a higher percentage. The other two ingredients viz aloevera and soymilk, were taken in such a manner that the body of the drink is not disrupted and blend well with the base ingredient.

Different proportions of ingredients were blended by "trial and error" method along with water to maintain the consistency. Various combinations tried out are presented in Table 1.

Table No. 1 Ratio of the constituents for the various combinations.

Combinations	Ingredients	Proportions
C_1	Honey : Aloevera pulp : soymilk : Water	5.0 : 2.0 : 1.5 : 1.5
C_2	Honey: Aloevera pulp : soymilk : Water	5.0 : 1.5 : 1.5 : 2.0
C_3	Honey : Aloevera pulp : soymilk : Water	5.0 : 1.0 : 1.5 : 2.5
C_4	Honey : Aloevera pulp : soymilk : Water	5.0 : 1.0 : 1.0 : 3.0
C_5	Honey : Aloevera pulp : soymilk : Water	4.0 : 1.0 : 1.0 : 4.0
C_6	Honey : Aloevera pulp : soymilk : Water	4.0 : 1.0 : 1.5 : 3.5
C_7	Honey : Aloevera pulp : soymilk : Water	3.5 : 1.0 : 1.5 : 4.0
C_8	Honey : Aloevera pulp : soymilk : Water	3.0 : 1.0 : 2.0 : 4.0
C_9	Honey : Aloevera pulp : soymilk : Water	2.5 : 1.0 : 2.5 : 4.0

Out of the nine combinations, two combinations were selected which were having similar scores. The best identified combinations were investigated in depth for the nutrient content, chemical constituents and storage stability.

3.3 Quality assessment of the product

Quality is the ultimate criteria of the desirability of any food product to the consumers. Sharma (2006) reported that quality is a very important parameter for judging the edible nature of any food product. Quality of the product was assessed in terms of chemical constituents, nutritional composition, viable count, and sensory quality and shelf stability using standard techniques.

3.3.1 Sensory evaluation of the formulations

3.3.1.1 Selection of judge panel

Sensory evaluation of the product was done using a panel of 10 trained and semi trained judges including the research scholars of KAU, Vellayani after administering duo trio test (Appendix I) prescribed for screening the judge panel.

3.3.1.2 Sensory Evaluation of the product.

Sensory evaluation plays an important role in acceptability of a new product. When the quality of the food is assessed by sensory organs, the evaluation is said to be sensory analysis (Simi, 2002). Manay and Swamy (2002) opined that sensory evaluation plays a vital role in the food industry because it represents very unique technique that harness human behavioral instincts of perception , learning, cognition, psychophysics and psychometrics for the evaluation of food quality.

Organoleptic evaluation is a scientific method that evokes, measures, analyzes the products as perceived through the senses of sight, smell and taste. All the combinations tried out were evaluated for its sensory properties using score on five point scale (Jellinick, 1985) and hedonic rating ISI (1971), (Appendix II and III).

3.3.2 Assessment of chemical and nutritional composition of the product

In the present study chemical constituents such as TSS, pH, titrable acidity, reducing sugar, total sugar and the nutrients such as carbohydrates, calories, protein, vitamin C, total minerals, sodium, potassium, calcium and iron were assessed using standard techniques.

3.3.2.1Chemical composition of the product

Chemicals constituents present in the food are potentially significant, as they determine the nutritional value, eating properties and suitability for use in particular products and processes (Huton, 2002). Chemical constituents viz, TSS, pH, titrable acidity, reducing sugars and total sugars in the selected combinations were ascertained using standard techniques. This will enable to identify and quantify the chemical components, as they will contribute to the quality,shelf stability and sensory properties to the product.

3.3.2.1.1 Total Soluble Solids (TSS)

TSS is solids that are dissolved within a substance. It is commonly used to measure sugar content in beverages and medicines.TSS of a probiotic drink was measured using Abbe refractometer and expressed in degree Brix(^{0} Bx)

3.3.2.1.2 PH

The pH value of a food is a direct function of the free hydrogen ions present in that food. The definition of pH as a measure of the free acidity of a food product. The pH of the probiotic drink was estimated using a digital pH meter. (Saini *et al*., 2001)

3.3.2.1.3 Titrable acidity

Titratable acidity is used to determine how acidic the product will taste. Acidity was determined by dissolving a known weight of sample in distilled water and then titrated against 0.01 N NaOH using Phenolphthalein as an indicator (Ranganna.2001)

3.3.2.1.4 Reducing sugar and Total sugars

Reducing sugars are sugars that have the hemiketal functional group somewhere in their molecular structure. The reducing sugars will be often expressed in terms of glucose since glucose is the most predominant reducing sugar present in fruits. Total sugars are also determined using the titration with Fehling's solution. The reducing and total sugars of the drink was estimated using the standard procedure.(Ranganna, 2001 and AOAC,2005)

3. 3.2.2 Nutrient Analysis

Nutrient content or nutrient density refers to the substances in food that give energy and improve health. It also refers to the amount of nutrients for the given volume of food (Michel, 2005). The developed product was analysed for carbohydrate, protein, vitamin C, calcium and iron. Nutrient analysis is necessary to know the nutrient content of the food. Nutrient analysis of the probiotic beverage was analyzed using the standard techniques. The following nutrients were determined in triplicates.

3.3.2.2.1 Energy

Energy is one of the macronutrient essential for the human beings to engage in some activities. Energy of the probiotic beverage was computed after determining the carbohydrate and protein content of the probiotic beverage

3.3.2.2.2 Carbohydrates

Carbohydrate is one of the important nutrient required for the energy to do work. Carbohydrate of the probiotic drink was estimated using anthrone reagent. (Sadasivam and Manikkam, 1992)

3.3.1.2.3 Protein

Protein is one of the most important nutrients required by the body to carry out a wide range of functions essential for the maintenance of life. Proteins are essential component of tissues and cells of the body (Gopalan *et al*., 2009) . Protein was determined by Micro- kjehdals method

3.3.2.2.4 Vitamin C

Ascorbic acid is a strong reducing agent and reduces the dye 2, 6 dichlorophenol indophenol gets converted to dehydroascorbic acid. Vitamin C of the probiotic drink was estimated titrimetrically using the dye 2, 6 dichlorophenol indophenol method. (Sadasivam and Manikkam, 1992 and AOAC, 2005)

3.3.2.2.5 Calcium

Calcium is essential for living organisms in particular in cell physiology .As a major material used in mineralization of bone, teeth and shells. Calcium is mainly obtained from cow's milk. Calcium of the probiotic drink was estimated by titration using EDTA. (Jackson, 1973).

3.3.2.2.6 Iron

Iron was estimated by using the method suggested by Jackson (1973).

3.3.2.2.7 Sodium

Sodium was estimated by using the method suggested by Jackson (1973).

3.3.2.2.8 Potassium

Potassium was estimated by using the method suggested by Jackson (1973).

3.3.2.2.8 Total Minerals

Total minerals were estimated by standard technique.

Table 2: Methods adopted for the determination of chemical/nutrient constituents of the beverage

Constituents	Methods
TSS	Rangannna (2001)
Titrable Acidity	Ranganna(2001)
Reducing Sugars	Ranganna (2001)
Total Sugars	Ranganna (2001)
pH	Saini *et al*., 2001
Carbohydrate	Sadasivam and Manikkam (1992)
Vitamin C	Sadasivam and Manikkam (1992)
Calcium	Jackson (1973)
Sodium	Jackson(1973)
Potassium	Jackson(1973)
Iron	Jackson (1973)
Proteins	Sadasivam and Manikkam (1992)
Calories	AOAC,2005

3.4 Probiotication process of the product

The probiotication process which was the key part of study carried out in following steps such as selection of inoculum, optimization of pH, dosage of inoculum, optimization of incubation period and pretreatment prior to probiotication.

3.4.1 Selection of inoculum

Lactobacillus acidophilus was the organism selected for the probiotication process. *Lactobacillus acidophilus* as a food or supplement has been the focus of studies for the last two decades (Denev, 2006). Hummel *et al.* (2007) reported that *Lactobacilli* are often considered to be commensal or beneficial participants in human microbial ecology and considerable research was being carried out on the effects for the use of lactobacilli as additives in human diets..

Rattanachaikunsopon and Phumkhachorn (2010) reported that *Lactobacilli* are highly competitive largely due to their applications in the production of fermented food. They can also produce antimicrobial substances including bacteriocins that have ability to inhibit pathogenic and food spoilage bacteria.

On the above grounds, in the present study *Lactobacillus acidophilus* was selected for the probiotication process

Sanz *et al.* (2005) reported that honey could enhance the growth of two probiotic bacteria viz. *Lactobacillus and Bifidobacteria* that are essential for better intestinal health. Vasudha and Mishra (2013) pointed out that soya milk is suitable for the growth of the lactic acid bacteria and the use of fermented soya milk drinks as probiotic is recommended by many researchers

3.4.2 Procurement of culture

Probiotic culture of *Lactobacillus acidophilus* (MTCC 10307) was procured from Microbial Type Culture Collection, Chandigarh (Plate 2).

3.4.3 Optimization of the pH and dosage of the inoculum

Procured culture of *Lactobacillus acidophilus* was freeze dried. For inoculation purpose, the culture was sub cultured in MRS (De Man Rogosa Sharpe) media. Culture was suspended into the broth at different pH ranging from 4.0 – 7.0.

Plate No 2: *Lactobacillus acidophilus*, Procured from MTCC, Chandigarh

Plate No: 3. Inoculation of culture into the beverage

Growth of the organism was monitored and based on the growth at different pH, pH suitable for the organism was identified.

After determining the pH of the culture, dosage of inoculum needed to obtain desired viable count in the product was standardized. For this purpose three dosages of inoculum viz.,1.0, 1.5 and 2.0 per cent were inoculated and incubated at different time intervals. Based on the desired viable count dose of the inoculum was fixed.

3.4.4 Optimization of the Incubation period

The beverage combinations rated best after the sensory analysis was selected for acidification with *Lactobacillus acidophilus*. The incubation period was optimized by inoculating the formulation at varying time intervals and recorded the viable count (plate 3).

Serial dilution and pour plate technique was employed to estimate the viable count. The procedure adopted for serial dilution was as follows: 1 ml of inoculated product was transferred into sterilized tube containing 9 ml sterile water and mixed well to obtain concentration becomes 10^{-1} dilution and 1ml from that tube was transferred to another tube 10^{-2} containing 9 ml sterile water to obtain 10^{-3} dilution. Likewise dilutions upto 10^{-8} and 10^{-9} were prepared.

Viable count of the product was assessed at an interval of 3 hours and also the possibility of the contamination was also assessed. Agar plates were stored at incubator at 37 ^{0} C for faster growth. As the growth of *Lactobacillus acidophilus* is very slow and viable count was recorded 48-72 hours of incubation period

3.4.5 Pretreatments prior to probiotication

Pretreatments are essential for improving the quality of the beverage. Pretreatments prior to the probiotication process was attempted to make the beverage consumer safe. Standardised beverage was subjected to sterilization at two different

temperatures at 100^0C and 80^0 C, with formulated beverage and also with the individual sterilized ingredients used.

3.4) Quality analysis of the probiotic beverage

Quality assessment of the probiotic product was done after 6 hours of incubation of culture (which is the best hour after inoculation) in terms of chemical constituents and nutritional components. Quality assessment of the probiotic drink was assessed to know how the probiotication affects the composition of the product. Quality of the probiotic beverage were analysed in terms of sensory, nutritional and chemical composition using the standard techniques.

3.5.1 Sensory evaluation of the probiotic beverage.

Sensory evaluation of product plays an important role in the development of a new product. The sensory evaluation of the probiotic beverage was carried out by trained and semi trained panel of 10 judges as explained in 3.3.1.2. Sensory evaluation of the product was done in comparison with non probiotic beverage maintained as control.

3.5.2 Assessment of chemical and nutritional composition of the product

Chemical constituents such as TSS, pH, titrable acidity, reducing sugar, total sugar and the nutrients such as carbohydrates, calories, protein, vitamin C, total minerals, sodium, potassium, calcium and iron were analysed after probiotication process in the beverage using standard techniques.

3.5.2.1.1 Total Soluble Solids (TSS)

TSS of the probiotic beverage was also assessed according to the method as mentioned in 3.3.2.1.1

3.5.2.1.2 PH

pH of the probiotic beverage was measured as mentioned in 3.3.2.1.2.**3.5.2.1.3 Titrable Acidity**

Titrable acidity of the probiotic beverage was analysed according to the technique described in 3.3.2.1.3.

3.5.2.1.4 Reducing Sugar and Total Sugars

The reducing sugars and total sugars of the probiotic beverage was measured according to the technique mentioned in 3.3.2.1.4

3. 5.2.2 Nutrient Analysis

The developed product was analysed for carbohydrate, protein, vitamin C, calcium and iron.

3.5.2.2.1 Carbohydrates

Carbohydrate content of the probiotic drink was analysed using the technique mentioned in 3.3.2.1.

3.5.2.2.2 Protein

The protein content of the probiotic beverage was analysed according to the method mentioned in 3.3.2.2.

3.5.2.2.3 Vitamin C

The vitamin C content of the probiotic beverage was also measured according to the method mentioned in 3.3.2.3.

3.5.2.2.4 Calcium

Calcium content of the probiotic beverage was also measured according to the method mentioned in 3.3.2.2.4.

3.5.2.2.5 Iron

Iron content of the probiotic beverage was measured according to the method mentioned in 3.3.2.2.5.

3.5.2.2.6 Sodium

Sodium content of the probiotic beverage was also measured according to the technique mentioned in 3.3.2.2.6

3.5.2.2.7 Potassium

Potassium of the probiotic beverage was measured according to the method mentioned in 3.3.2.2.7

3.5.2.2 .8 Total minerals

Total minerals of the probiotic beverage was also measured according to the method mentioned in 3.3.2.2.8.

3.6 Shelf stability of the probiotic beverage.

According to Shankar (2000) several factors such as raw material quality, storage temperature, storage containers, processing methods, the environment in which it is processed etc will affect the microbiological quality of the food.

The probiotic beverage was prepared in large quantity under hygienic conditions and stored in glass bottles and stored at ambient (30 ± 1^0 C) and refrigerated conditions (4 ± 1^0 C) . Changes in sensory characteristics, total viable counts of *Lactobacillus acidophilus* and Coliforms, if any pH, titrable acidity and total sugars were studied during storage. Quality assessment of the product stored at the ambient condition was done on everyday till it deteriorates. For the refrigerated product quality was ascertained on alternate days till the beverage spoiled.

3.7) Consumer Acceptability of the probiotic beverage.

Consumer acceptance of any new innovative food products plays an important role in its marketing. Food firms interested in using innovativeness as a competitive strategy have to constantly analyse the change in their target-consumer perceptions, tastes and preferences (Shah, 2007).

The consumer acceptance of the developed beverage was subjected to consumer acceptance test using hedonic rating on nine point scale. Thirty consumers comprising 10 school children, 10 college students and 10 professionals were selected at random for the purpose.

3.8 Cost and Yield of the product.

The cost and yield of the beverage was carried out by accounting the cost of all the ingredients used for the formulation of beverage including the culture (inoculum) and 10 percent overhead charges was also added as fuel, electricity and labour involved.

3.9 Statistical analysis

In order to obtain suitable interpretation, the generated data was subjected to statistical analysis. Kruskal wallis, ANNOVA and Students t test.

RESULTS

4. RESULT

The results of present study entitled "Development and quality evaluation of probiotic honey beverage" are detailed in this chapter under the following heads:

4.1 Product formulation and standardization.
4.2 Quality assessment of the beverage.
4.3 Probiotication process of the beverage.
4.4 Quality assessment of the probiotic beverage.
4.5 Shelf stability of the probiotic beverage.
4.6 Consumer acceptance of the probiotic beverage.
4.7 Cost and yield of the probiotic beverage.

4.1 Product formulation and standardisation

Standardisation and product development play a key role in the growth of food industries. According to Poduval (2002) one of the foremost purposes of standardization is to facilitate the movement of materials and products through all stages of production in any industrial activity starting from the raw material to the finished products, then to the dealer and finally to the retailers and consumers . Sohrab (2000) opined that standardization encapsulates technological results and becomes a vehicle for technology transfer while quality is the key for facilitating trade and satisfying consumers

4.1.1 Standardisation of the beverage

4.1.1.1 Optimization of ingredients

The major ingredients used for the formulation of the drink were honey, aloevera and soymilk. The selected ingredients were blended in different proportions to obtain an acceptable honey based beverage

The proportions of ingredients required for the formulation of the beverage was determined by blending the selected ingredients through "trial and error method" and evaluating its sensory quality. Altogether twelve combinations were worked out and subjected to sensory evaluation by expert panel of judges. Out of the

various combinations worked out nine combinations were come out successfully with proper body and consistency (Plate 4).

4.1.1.2 Sensory evaluation of the combinations tried out.

Sensory analysis is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses viz sight, smell, taste, touch and hearing for the purpose of evaluating consumer preference (IFT, 2005). Although sensory evaluation of foods is the most important quality assessment, taste evaluation is not practical for routine quality control. It is always preferable to have a quantitative method for which rejection points may be established by sensory means (Jonnalagadda *et al*., 2001).The discipline requires panels of human assessors, on whom the products are tested, and recording the responses made by them. According to Simi (2002) when the food is assessed by human sensory organs, the evaluation is said to be sensory analysis. Numerical scoring is used to evaluate particular characteristics of one or more samples indicating the rating as excellent, very good , fair and poor (Manay and Swamy,2000) .The organoleptic evaluation of product was done by a panel of 10 judges using a 5 point scale and the data is presented in Table 3.

Plate No: 4. Nine combinations tried out

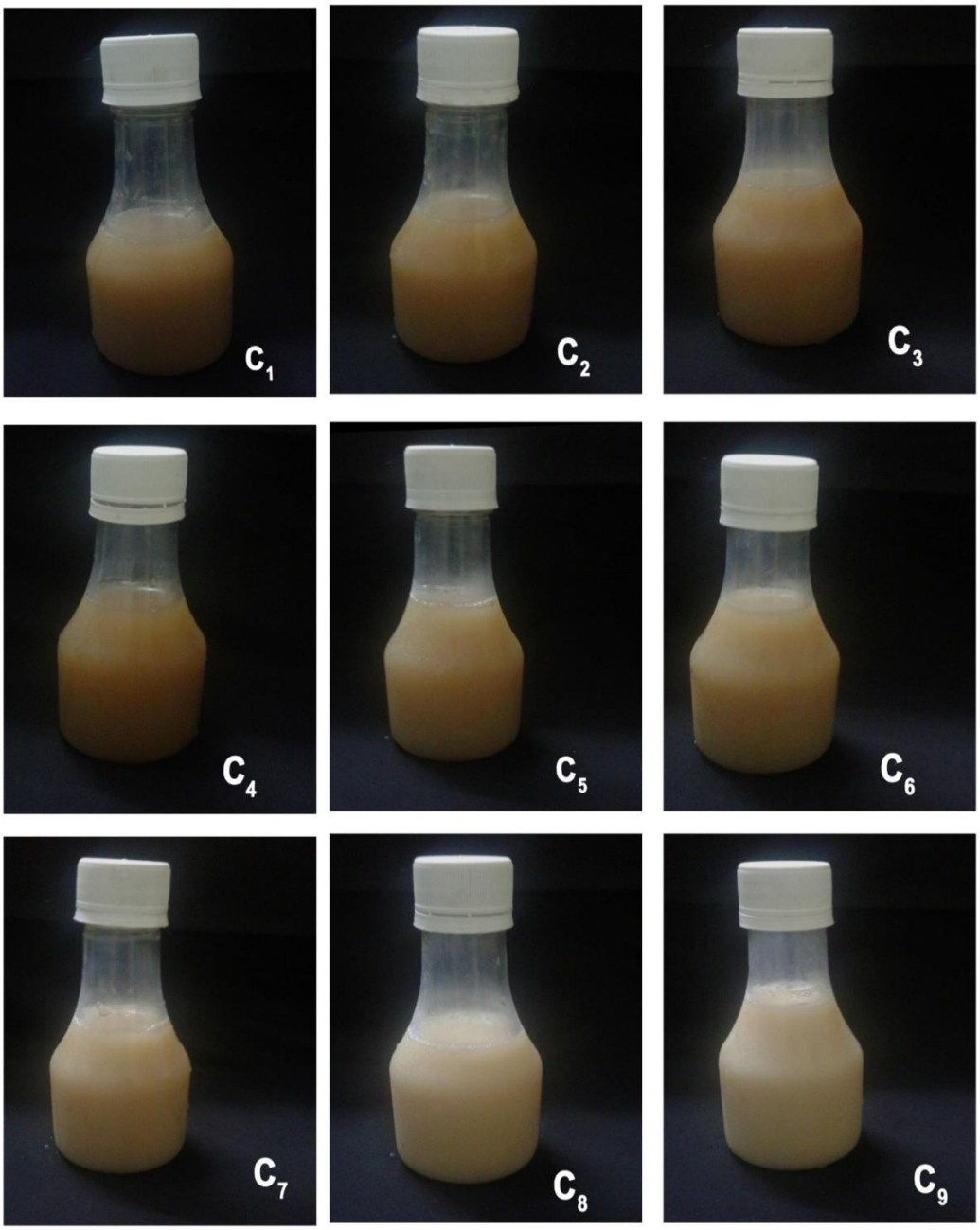


Table 3: Mean rank scores obtained for sensory analysis of the developed combinations tried out

Combinations	Appearance	Colour	Flavour	Taste	Consistency	Overall acceptability
C_1	39.3	34.9	33.6	31.4	27.4	33.4
C_2	34.0	27.6	30.2	27.8	34.6	31.8
C_3	30.9	38.5	37.1	24.2	29.4	32.2
C_4	36.3	38.5	33.8	31.4	38.2	35.2
C_5	50.8	52.3	47.9	55.1	41.8	55.5
C_6	**55.0**	**55.2**	**58.4**	**61.5**	**58.4**	**59.0**
C_7	36.3	34.5	37.2	35.0	38.2	34.6
C_8	**59.2**	**58.8**	**62.0**	**70.1**	**68.5**	**66.0**
C_9	**67.6**	**69.0**	**69.2**	**72.9**	**75.0**	**69.5**
K value	26.70	27.18	30.4	50.7	43.51	39.82
C.D (0.05)	21.73					

Appearance

The surface characteristics of food products contribute to the appearance. The quality of a food item may simply be judged from its appearance when it is placed in front of a consumer. The first impression of a food is usually visual and a major part of willingness to accept a food depends on its appearance.

According to Srilakshmi (2010) the appearance of the food products is contributed by surface characteristics viz., size, shape, colour, transparency, opaqueness, turbidity, dullness etc.

The mean ranks for appearance pertaining to the nine combinations tried out ranged between 30.9- 67.6.The highest mean rank (67.6) was obtained for C_9 which was having equal proportions of honey and soymilk. The mean rank for combinations C_8 and C_6 were on par with C_9 with mean ranks of 59.2 and 55.0 respectively. The mean ranks for C_1, C_2, C_4, C_5 and C_7 were 39.3, 34.0, 36.3, 50.8,

and 36.3 respectively. The least mean rank was obtained for C_3 (30.9) combination. Analysis of scores revealed that the formulation C_9 was judged to be the best in appearance score. Appearance of the beverage was found to be similar to the commercially available flavored milk.

Colour

Colour is used as an index to the quality of a number of foods. Colour is one of the important visual attribute that has been used to judge the overall quality of foods for a very long time. If the colour is unattractive, a potential consumer may not be impressed by any other attributes.

In addition to giving pleasure, the colour of food is associated with other attributes (Srilakshmi, 2010). Among the formulations, C_9 secured the highest score (69.0) and the combinations C_8 (58.8) and C_6 (55.2) were on par with C_9, while C_2 (27.6) recorded the least mean rank for colour attribute. The scores for other formulations were C_1 (34.9), C_3 (38.5), C_4 (38.5), C_5 (52.3) and C_7 (34.5) respectively. Significant difference was noted in various proportions tried out. Colour of the formulated beverage could be compared with the commercially available flavoured milk. The colour of the formulated combination varies from cream to brown. The colours of the formulations vary with the quantity of honey and soymilk added to it.

Flavour

Evaluation of flavour factor is highly subjective and depends on the discriminating ability of the consumer as flavour includes the senses of smell as well as the senses of taste as experienced by a consumer. Flavour is commonly defined as being the sensation arising from the integration or interplay of signals produced as a consequence of sensing smell, taste and irritating stimuli from a food or a beverage (Shankaracharaya, 2002).

Flavour characteristics may be evaluated by instrumental methods as well as by panel of judges. The flavour of food has three components – odour, taste,

and a composite of sensations known as mouth feel (Srilakshmi, 2010). Odour preference is generated by stimulation of sensory cells by specific volatile compounds present in food. The flavour of the developed product is due to the natural flavour of the ingredients used.

As per the flavour evaluation, flavour of the different combinations differed in mean ranks, which ranged from 30.2- 69.2. The highest mean rank was recorded by C_9 (69.2) while the combinations C_8 (62.0) and C_6 (58.4) were on par. The least mean rank was secured by C_2 (30.2).The mean ranks of other formulations were C_1 (33.6), C_3 (37.1), C_4 (33.8), C_5 (47.9) and for C_7 (37.2) respectively. Significant differences were noticed in the flavour attribute of the various combinations. The flavour of the developed formulations was pleasant and similar to commercially available flavoured milk .

Taste

The taste is the major attribute which determine the acceptability of a food. Taste is the sensation produced when a substance in the mouth reacts chemically with receptors of taste buds. According to Srilakshmi (2010) taste sensation which the taste buds register are categorized as sweet, salt, sour or bitter.

The taste of each formulation was assessed and their scores were recorded. The mean ranks for taste parameter in the nine combinations ranged from 24.2- 72.9. Maximum mean rank for taste was obtained for C_9 (72.9) the combinations C_8 (70.1) and C_6 (61.5) were on par with C_9 .C_3 combination recorded the least mean score of 24.2. The mean ranks obtained for the other formulations were C_1 (31.4), C_2 (27.8), C_4 (31.4), C_5 (55.1) and C_7 (35.0) respectively. The differences in the taste scores in the nine proportions tried out were found to be statistically significant. All the formulations were having the taste similar to the aerated drinks. Sweetness of the beverage varies according to the quantity of honey used in the formulations and decreases when the quantity of honey in the formulations was reduced.

Consistency

Consistency constitutes the physical property of the food stuff as apprehended by the eye, skin and muscle senses located in the mouth. As it is a beverage/drink its texture can be valued in terms of their consistency or fluidity.

The scores obtained for consistency for different combinations varies. As mentioned in the methodology, water is added to the formulations for getting proper consistency. The highest mean ranks for consistency was also secured for the C_9 combination (75.0). Next to C_9 was C_8 and C_6 with the mean ranks 68.5 and 58.4 respectively while the combination C_1 (27.4) recorded the least mean rank. The mean ranks recorded for other combinations like C_2, C_3, C_4, C_5 and C_7 were 34.6, 29.4, 38.2, 41.8 and 38.2 respectively. Significant difference was observed in the scores for consistency of the beverage at 5% level. The consistency of all the formulations differ and it depends on the proportion of water added to the different formulations.

Overall Acceptability

The overall acceptability of the formulations depends on the sum total of the scores obtained for the parameters viz performance of the beverage on the whole, considering the judges perception on different sensory attributes.

The overall mean ranks for different combinations ranged from 31.8- 69.5. Over all acceptability scores clearly depicted that among the nine formulations, maximum mean rank was secured for C_9 (69.5) combination followed by C_8 (66.0) and C_6 (59.0). Least preference was obtained for C_2 (31.80). Overall acceptability mean ranks of different formulations were C_2 (31, 8), C_3 (32.2), C_4 (35.2), C_5 (55.5) and C_7 (34.6) respectively. Significant difference was found between the combinations with respect to over all acceptability at 5 percent level.

On the basis of analysis of mean ranks obtained C_9 (69.5) was selected as the best combination followed by C_8 (66.0) and C_6 (59.0).

4.1.1.2.1 Hedonic rating of the Formulations.

The hedonic rating is used to measure the consumer acceptability of food products (Srilakshmi, 2010). Hedonic rating was also carried out for all the nine combinations on nine point scale from 'like extremely' to 'dislike extremely'. The scores obtained by hedonic rating are presented in the Table 4.

Table 4: Hedonic rating of the formulations

Combinations	**Scores Obtained**
C_1	6.3
C_2	6.4
C_3	6.2
C_4	6.4
C_5	7.2
C_6	7.3
C_7	6.8
C_8	8.1
C_9	**8.4**

As indicated in the table, on the hedonic rating also C_9 (8.4) combination found outstanding followed by C_8 (8.1) and C_6 (7.3).Hence these three combinations were selected for probiotication process (Plate 5).

Table 5: Proportion of ingredients used in the selected formulations.

Combinations	Honey(ml)	Aloevera pulp(ml)	Soymilk (ml)	Water(ml)	Proportion (H:A:S:W)
C_6	40	10	15	35	4:1:1.5:3.5
C_8	30	10	20	40	3:1:2:4
C_9	25	10	25	40	2.5:1:2.5:4

The above table indicated the quantity and proportion of ingredients used in the formulation of the beverage. In the selected three combinations, quantity of honey and soyamilk varies with the combination; while quantity of aloevera

Plate No: 5. Selected combinations

remains same in the three combinations. C_9 was found to be more acceptable than the other two combinations. It was found that the acceptability of the beverage increases with the quantity of soya milk added in the formulation.

Table 6: Sensory evaluation of flavour enhancement of the selected combinations.

Combinations	F_1	F_2	F_3	F_4
C_6	**20.1**	11.1	10.7	12.1
C_8	16.6	15.3	16.7	13.7
C_9	9.8	**19.1**	**20.1**	**20.7**
Kvalue	8.72	7.00	6.28	**6.77**
C.D(0.05)	11.49			

F_1- Lime emulsion, F_2- Chocolate, F_3- Vanilla essence, F_4- Cardamom essence

After obtaining the specified proportion, it is imperative to improve the flavour of the beverage to enhance consumer appeal. The developed beverage maintained natural flavour which was attributed to the three ingredients used in the formulation. In the selected three combinations, four different flavours such as lime emulsion, chocolate, vanilla and cardamom were added. The flavoured formulations were subjected to sensory analysis, scores of which are depicted in the Table (6).

Scores secured by the three combinations when lime emulsion was added were C_6 (20.1), C_8 (16.6) and C_9 (9.8) respectively. Presence of lime flavour resulted in curdling of the beverage which was having greater amount of soyamilk. So C_9 scored the least. The liquid chocolate flavour when incorporated, C_9 scored highest (19.1), followed by C_8 (15.3) and C_6 (11.1). The third flavour tried was vanilla which is the commonly used flavour in the beverage. In this flavour also C_9 (20.1) secured maximum. The scores obtained by C_8 and C_6 are 16.7 and 10.7 respectively.

Cardamom flavour incorporated beverages were adjudged to be most appealing to the judges with the scores C_9 (20.7), C_8 (13.7) and C_6 (12.1)

respectively. Since the flavour of drink is one of the important criteria for the acceptance by the consumers, it is decided to flavour the beverage with cardamom which could be comparable with commercially available flavoured milk.

Before proceeding to the probiotication process quality analysis of the three combinations were carried out. Results of which are presented in the following section.

4.2. Quality assessment of the selected combinations

Sivasankar (2013) pointed out that quality of foods and food products may be defined as the degree of excellence of the various characteristics that influence consumer acceptance as well as consumer safety. Quality assessment in terms of its chemical constituents is highly essential for the stability of a drink. The chemical constituent also helps to enhance the shelf life of the product. Hence for the best three combinations were subjected to chemical and nutrient analysis.

The chemical and nutrient analysis of the beverages was carried out as described in the methodology in triplicates. The chemical constituents such as TSS, pH, acidity, reducing sugars and total sugars and the nutrient constituents such as carbohydrates, protein, vitamin C, energy, iron , sodium , potassium, calcium and total minerals were the components ascertained. The mean of the triplicates are tabulated in the table.

4.2.1 Chemical analysis

Sivasankar (2013) reported that Chemical and instrumental methods are used for evaluating the nutritional quality.

Chemical constituents of the three combinations viz TSS, pH, acidity, reducing sugars and total sugars are presented in Table 7.

Table 7: Chemical analysis of the selected combinations.

Beverage	TSS(^{0}brix)	pH	Titrable acidity (%)	Reducing sugars (g of glucose/100g of juice.)	Total sugars(g of glucose/100 g of juice)
C_9	**23**	**6.5**	0.068	29.40	78.11
C_8	20	5.5	0.074	26.55	73.09
C_6	22	4.4	**0.080**	**32.0**	**93.98**
C.D(0.05)	NS	NS	0.002	1.018	0.592

4.2.1.1 TSS

Total soluble solids in a food combination will determine the sugar content as well as contribute to the taste of the product.

TSS of the combination C_9 was 23^0 brix followed by C_6 and C_8 with 22^0 brix and 20^0 brix respectively which indicated more or less similar sweetness in the beverages.

4.2.1.2 pH

pH is one of the other important chemical constituent in the food which trigger and affect the growth of microorganisms in a product. Balaji and Prasad (2014) viewed that the pH has great importance to maintain shelf stability; pH can also influence the flavor and processing requirements of any beverage.

The pH of the beverage was recorded as 6.5, 5.5 and 4.4 respectively in C_9, C_8 and C_6. C_9 and C_8 were towards the alkaline while C_6 was more towards acidic. No significant difference was noted in the pH of the beverages.

4.2.1.3 Acidity

Acidity is one of the prime chemical constituents which indicate the deteriorative changes in the product. Balaji and Prasad (2014) reported that acidity is

also an important attribute because tartness is a major factor in the acceptability of RTS beverage.

As indicated in the table, C_9 combination is having acidity of 0.068%, while acidity of C_8 and C_6 were 0.074% and 0.080% respectively. Significant difference was noticed in the acidity of the beverages at 5 per cent level.

4.2.1.4 Reducing sugars

Srilakshmi (2010) reported that sweetness appears to be associated with the hydroxyl radicals on the sugar molecules. Some sugars act as reducing agents and such sugars will contain an aldehyde functional group. This property can be used as a basis for the analysis of reducing sugars (Thorpe, 2001)

Reducing sugar content of the product will be expressed in terms of glucose as it is the most predominant sugars in the drinks. Some of the reducing sugars are glucose, galactose, lactose and maltose (Sadasivam and Manikkam, 1992).

Comparing the reducing sugar content of the selected combinations, it is noted that C_6 recorded higher reducing sugar content 32.0 g of glucose/100g of juic)e than C_9 (29.40 g of sugar/100 g of juice) and C_8(26.55 g of glucose/100g of juice). Statistical analysis revealed significant difference in the reducing sugar content of the three combinations at 5 per cent level.

4.2.1.5 Total Sugars

Total sugar content of the selected combinations determined were comparatively high and C_6 recorded highest sugar content of 93.98 g of glucose/100g of juice followed by C_9 (78.11 g of glucose/ 100 g of juice) and C_8 (73.09 g of glucose/100 g of juice. Statistical analysis also revealed that there was a significant difference in the total sugar content of the combinations tried out at 5 per cent level.

4.2.2) Nutrient analysis of the selected combinations.

Food may be defined as substances, which when eaten and absorbed by the body, maintain life and growth. The chemical components that perform these functions are called nutrient. Nutritional quality of a food or food product may be

evaluated by specific analysis for essential nutrients such as proteins, vitamins, minerals and other nutrients (Sivasankar, 2013).The major nutrients analysed were Energy, Carbohydrates, Viatmin C and minerals.

Table 8: Nutrient analysis of the selected combinations.

Combinations	Energy (Kcal)	Carbohydrates (g/ 100 g of sample)	Protein (g/100g of sample)	Vitamin C(mg/100 g of sample)
C_9	256	64.66	0.48	0.063
C_8	291	72.66	**0.81**	0.072
C_6	**326**	**81.33**	0.65	**0.094**
C.D(0.05)	7.99	1.99	0.003	0.987

4.2.2.1 Carbohydrate

Carbohydrate is one of the major biologically essential molecules found in all living organisms. They are used as main form of energy store house. They are responsible for the production and regulation of blood glucose. The carbohydrate composition in foods influence flavour, texture and colour and thereby the acceptability of foods (Sivasankar, 2013)

Table 8 represents the carbohydrate content of the three selected combinations. C_6 combination has got maximum carbohydrate content of 81.33 g of carbohydrate/100 g of sample while C_8 (72.66 g of carbohydrate/ 100 g of sample) and C_9 (64.66 g of carbohydrate/100 g of sample). Significant difference was observed in the carbohydrate content of the selected combinations.

4.2.2.2 Protein

Proteins are of importance in human food with respect to two aspects namely nutrition and textural quality of food. Some are involved in the structural support and movement; others in enzymatic activity. Proteins and their hydrolytic products, peptides and free amino acids contribute to the flavour and taste of foods (Sivasankar, 2013).

The protein content of the selected samples are presented in the Table 8.It was clear from the table that the protein content of selected combination were comparatively low and differs each other. Protein content of C_9, C_8 and C_6 were 0.4g%, 0.8g% and 0.6g% respectively with a significant difference at 5 per cent level.

4.2.2.3 Energy

A calorie is a measure of how much energy that the protein, carbohydrates, and fat can supply to the body. Food plays a vital role in providing the body energy for functions such as breathing and physical activity.

Energy content of the three combinations of the beverages as revealed in the table indicated highest energy value of 326 kcal in C_6 combination while C_9 recorded least caloric value of 256 kcal and C_8 had an energy value of 291 kcal. Significant difference was observed at 5 per cent level.

4.2.2.4 Vitamin C

It was found that vitamin C content of the beverages was negligible and does not contribute much from the beverage. Vitamin C content recorded in the C_9, C_8 and C_6 were 0.063mg/100g,0.072mg/100g and 0.094mg/100g respectively and on par with each other. Significant difference was noticed in the vitamin C content at 5 per cent level.

4.2.3 Mineral content of the beverage.

Minerals are one of the important parts of all the foods especially in beverages. The minerals/ trace elements like iron, sodium, potassium and calcium of the selected formulations were found out using calorimeter, Atomic Absorption Spectrometry and volumetric method.

Table 9: Mineral content of the selected combination

Product	Iron (mg/100g)	Sodium (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Total Minerals (%)
C_9	0.25	1.1	0.4	**280**	2.5
C_8	0.59	1.5	0.5	260	2.0
C_6	**0.87**	**2.3**	**0.8**	230	1.5
C.D(0.05)	2.069	0.207	-	0.262	-

4.2.3.1 Iron

The minerals present at levels less than 0.05 percent in the human body are defined as micro minerals. Iron is a necessary trace element found in nearly all living organisms. It helps to metabolize proteins and plays a role in the production of hemoglobin and red blood cells. (Srilakshmi, 2010)

Mineral content of the formulations was analysed and represented in the Table 9.It is clear from the table that C_8 (0.059 mg/100g) has got more iron content than the C_9 (0.25mg/100g). Statistical analysis also revealed that there was a significant difference at 5 per cent level.

4.2.3.2 Sodium

Table 9 shows the sodium content of the selected combinations. Sodium content of the formulations was almost similar and very low. Sodium content of C_9 (1.5mg/100g), C_8 (1.1 mg/100 g) and C_6 (2.3 mg/100 g) respectively. Statistical analysis revealed that there was a significant difference at 5 per cent between the three combinations.

4.2.3.3 Potassium

Table 9 shows the potassium content of the selected formulations. Potassium content of the combinations was negligible. Potassium content of C9, C8 and C_6 were 0.4mg/100g, 0.5mg/100g and 0.8 mg/100g respectively. No significant difference was observed in the potassium content.

4.2.3.4 Calcium

Calcium is essential for living organisms, in particular in cell physiology. Calcium is the most abundant mineral in the body .Body uses 99 percent of calcium to keep the bones and teeth strong, thereby supporting skeletal structure and function. The rest of the calcium in the body plays key roles in cell signaling, blood clotting, muscle contraction and nerve function.

Table 9 shows the calcium content of the three combinations. C_9 (280 mg/100g of sample) has got more calcium content than C_8 (260 mg/100 g), while C_6 has got calcium content of 230mg/100g. Statistical analysis revealed that there was a significant difference in calcium content at 5 per cent level.

4.2.3.5 Total Minerals

The total minerals of the three selected combinations were recorded. The total minerals of the formulations were ascertained using the ash content. Total mineral content of the formulations C_6, C_8 and C_9 are 2.5%, 2.0% and 1.5% respectively.

Table 10: Combinations selected for the probiotication process

Combinations	**pH**
C_9	6.5
C_8	5.5

Honey- H, Aloevera Pulp- A, Soya Milk-S,Water-W.

From the results obtained it is inferred that three combinations of the beverages were consumer acceptable and nutritionally excellent. However the pH of the combination recorded were 6.5, 5.5 and 4.4 respectively for C_9, C_8 and C_6.For probiotication process, low pH beverage was not preferred as it will not support the growth of organism. Hence the combination C_6 was discarded and the other two combinations were selected for the probiotication process.

4.3 Probiotication Process of the beverage.

According to FA0 (2003) probiotics are live microorganisms that when consumed in enough amounts exert health benefits. In recent years, several authors have studied about fermentation of different fruit juice by probiotic lactic acid bacteria (Yoon, 2006).

According to Champagne *et al* (2005) several factors that affect the survival of probiotics are type and form of the culture, the amount of bacteria required to obtain a beneficial effect on viability, the determination of probiotic cells in the product, stability during storage and possible changes in the sensory properties. Corrales *et al* (2007) reported compatibility and adaptability between the selected strain and food used as a carrier is fundamental of a probiotic product.

4.3.1 Selection of culture

In the probiotication process, selection of microorganism is very important. The most commonly used probiotic microorganisms in foods for human consumption are *Lactobacillus* and *Bifidobacterium* sp. which have depicted significant health benefits associated with ingestion of microorganisms (Stanton *et al.*, 2001).

In the present investigation *Lactobacillus acidophilus* was used as the culture for the probiotication process.

4.3.2 Preparation of culture and optimization of pH

Freeze dried culture of *Lactobacillus acidophilus* was procured from MTCC, Chandigarh. One loop full of culture was inoculated into MRS broth (150 ml) having different pH. The Table 11 shows the growth of culture in media with different pH.

Table 11: Presence/ absence of growth of *Lactobacillus acidophilus* in different pH.

pH	Presence/ Absence of Growth of *L. acidophilus*
4.0	Absent
4.5	Absent
5.0	Absent
5.5	Absent
6.0	Absent
6.5	**Present**
Reconfirmation of growth of *L.acidophilus* in different pH	
6.1	Absent
6.2	**Present**
6.3	**Present**
6.4	**Present**
6.5	**Present**
6.6	**Present**
6.7	Absent

The presence/ absence of growth was found out by streaking broth in MRS media. It can be noted from the table that no growth was detected at a pH from 4.0 - 6.0. While at a pH of 6.5 the presence of growth was visible in the broth and also when streaked in MRS agar plates. In order to reconfirm growth of the culture, it was again inoculated at pH range of 6.1- 6.6 .Results clearly revealed that growth of the organism was observed in a pH range of 6.2-6.6.

Kechagia *et al.* (2013*)* reported that probiotic products should have a maximum concentration of 10^6 cfu/ml or g and that a total of some 10^8 to 10^9 cfu/ml probiotic microorganisms should be consumed daily for the probiotic effect to be

transferred to the consumers. This point was considered while adopting the probiotication process.

4.3.3 Optimization of dosage of inoculum

After determining the pH of the culture, percentage of inoculum needed to obtain desired viable count in the product was to be standardized. This was carried out by inoculating different doses of culture at varying time intervals viz 3 hours, 6 hours and 9 hours. In the present investigation for this purpose three levels of inoculum viz 1.0%, 1.5% and 2.0% were inoculated with the beverage. Results are presented in Table 12. Five replications were maintained for each level of inoculum.

Table 12: Viable count of *Lactobacillus acidophilus* at varying time intervals and doses.

Dosage of inoculums (%)	(x 10^9 cfu/ ml)		
	3 hrs	6 hrs	9 hrs
1.0	**46.0**	**85.8**	**64.4**
1.5	64.0	115.0	96.0
2.0	75.0	135	106

Viable count of 1% inoculum at 3hours of incubation was 46x10^9cfu/ml, while 85.8 x 10^9cfu/ml at 6 hours of incubation and 64.4 x 10^9 cfu/ml at 9 hours of incubation. With respect to 1.5% inoculum, viable count at 3 hours of incubation was 64x 10^9 cfu/ml, at 6 hours of incubation 115x 10^9 cfu/ml and at 9 hours of incubation was 96x 10^9 cfu/ml.Viable count recorded with 2% inoculum at 3 hours, 6 hours and 9 hours of incubation was 64.4x10^9cfu/ml, 96x 10^9cfu/ml and 106 x10^9 cfu/ml respectively .

Maximum viable count was recorded at 6 hours of incubation with different doses of inoculum (Table 12). There after the count decreased. Since the prescribed level of viable count in the probiotic beverage suggested was 10^6 10^9 per

ml, 1% inoculum at 6 hours of incubation itself attained the desirable level of viable count. Therefore in present study 1% inoculated and at 6 hours of time period was selected for the preparation of the probiotic beverage (Plate 6).

4.3.4 Pretreatment prior to probiotication.

Sterilization is a method of heat treatment given to products at specific temp (100^0C, 80^0C) in an autoclave for three consecutive days. The sterilization was done to the beverage to avoid any contamination in the beverage and to make it consumer safe. Sterilization was attempted at two different temperatures.

Table 13: Viable count of *Lactobacillus acidophilus* (Sterilization at 100^0C and 80^0 C)

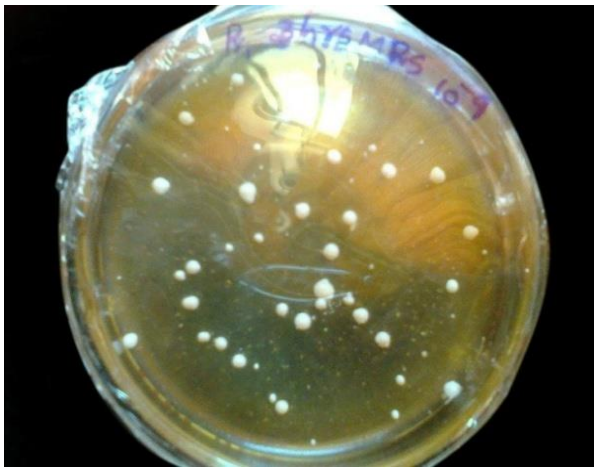
Pretreatments	3Hrs	6 Hrs	9 Hrs
	x10^9 cfu/ml	x 10^9 cfu/ml	x10^9 cfu/ml
Sterilization (100^0 C)	33.0	**74.4**	53.6
Sterilization (80^0 C)	44.6	**84.4**	63.6

Table 13 shows the viable count of the sterilized beverage after inoculation with 1% inoculum, at varying incubation period.

When the beverage was subjected to sterilization at 100^0 C, the colour/appearance of the formulation was changed and curdling occurred in the beverage. The viable count of Lactobacillus *acidophilus* depicted maximum count of 74.4 x 10^9 cfu/ml at 6 hours and least at 3 hours (33x10^9 cfu/ml) of incubation period while nine hours of incubation, decreased the count drastically to 53.6x 10^9 cfu/ml

Since curdling appeared in the beverage, high temperature was not suitable for the beverage as pre processing hence subjected to sterilization at a lower temperature of 80^0 C. At this temperature, though curdling was not there the colour/appearance of the beverage was altered slightly. The viable count of

Plate No: 6. Growth of *Lactobacillus acidophilus* at various incubation period



3 Hours

6 Hours

9 Hours

Lactobacillus acidophilus was maximum at 6 hours (84.4 x 10^9 cfu/ml) and least viable count was observed at 3 hours (44.6 x 10^9 cfu/ml) and viable count of 9 hours was 63.6 x 10^9 cfu/ml. Results indicated that heating of the beverage at two different temperatures, was found to reduce the growth of organisms and the consumer appeal of the product.

This has necessitated the need for individual ingredients to be sterilized independently. Heating of honey causes the production of toxic substances in the product. Hence it was decided to sterilize aloevera pulp and water separately before the probiotication process (Plates 7).

Each ingredient viz, honey, soyamilk, aloevera pulp and water used was streaked in EMB agar plates in order to ensure that it is free from pathogenic organism. Coliforms was not found in honey, soymilk while aloevera pulp, indicated presence of coliforms. Hence aloevera pulp and water used for the formulation of the beverage, sterilized in autoclave at 121^0 C and 15 P.S.L pressure and streaked in EMB agar plates and no contamination was detected. Thus with sterilization, ingredients except honey and soymilk, probiotic beverage was standardized. Viable count was recorded and presented in Table 14.

Table 14: Viable count of *Lactobacillus acidophilus* in the product after Inoculation at interval of 3 hours.

Probiotic beverage	3Hrs	**6 Hrs**	9 Hrs
	x 10^9 cfu/ ml	**x 10^9 cfu/ ml**	x 10^9 cfu/ ml
Honey, Soyamilk, Sterilized Aloevera pulp and sterilized water	56	**95.8**	74.4

It was observed that 6 hours incubation recorded highest viable count (95.8 x 10^9 cfu/ml) and least was noted at 3 hours. The viable count increased from the time of inoculation up to 6 hrs and decreased after 24 hrs. The viable count recorded was found to decreased with increase in temperature. With respect to the

Plate No: 7. Sterilized Ingredients

sterilization of the individual ingredients, the count increased which favour the growth of *Lactobacillus acidophilus* .

Table 15 : Count of *Coliforms* in the product after inoculation (x 10^7 cfu/ml)

Sample	3 Hours	6 Hours	9 Hours
Probiotic Beverage	Nil	Nil	Nil

Presence of any pathogenic organisms was checked and data is presented in Table 15. No pathogenic organism was detected in the probiotic beverage. Thus the probiotic beverage was standardized.

4.4 Quality assessment of the probiotic product

Quality assessment of the probiotic beverage was carried in terms of sensory, nutrient and chemical constituents.

4.4.1 Sensory evaluation of the probiotic product

According to Thakkar and Shah (2009) sensory analysis is a technique that uses man as a measuring instrument.

While conducting the sensory evaluation, non probiotic beverage was maintained as control for comparison (Plate 8). The results of the organoleptic/ sensory evaluation of the product are presented in the Table 16.

Table 16: Average score of the sensory attributes of the probiotic beverage

Beverage	Appearance	Colour	Flavour	Taste	Consistency	Overall acceptability
Probiotic Beverage	**4.6**	**4.7**	**4.6**	**4.5**	**4.7**	**4.5**
Non Probiotic Beverage	4.1	4.1	4.1	4.0	4.1	4.0
t value	3.16*	4*	3.16*	2.5*	3.26*	3.16*

Significant at 5%- * P<0.05,

Plate No: 8. Probiotic beverage v/s Non Probiotic beverage

Appearance

The scores obtained for the parameter appearance of probiotic and non probiotic are 4.6 and 4.1 respectively. By the probiotication process, the general appearance of the beverage significantly enhanced at 5 percent level.

Colour

Colour is an important factor in the acceptance of a product. There was a slight difference in the colour of the probiotic beverage in comparison with the control. The average score obtained for colour of probiotic beverage was 4.7 as against 4.1 in the control beverage. The colour of beverage becomes slightly darker than the control and was more appealing.

Flavour

Bajaj *et al*. (2002) found that flavour imparts recognizable character to the food products. As indicated in the table probiotic beverage has recorded an average score of 4.6 and that of non probiotic was 4.1. Significant difference at 5 percent level was found in the beverage with respect to flavour. The flavour of the beverage has altered with the probiotication process and has the flavour of the beverage was similar to the cardamom milk available in the market.

Taste

Assessment of the taste is an important attribute in the acceptance of a new product. Probiotic beverage revealed that has recorded more acceptances (4.5) compared to non probiotic beverage (4.0). The members of panel expressed that the taste of beverage imparted slightly tart taste and was highly acceptable to the judges. Significant difference was noticed in the taste attribute of the beverage at 5 percent level.

Consistency

Assessing the consistency of the two combinations, it was found that probiotic beverage was more thicker and cloudier than the control and scored 4.7 as against 4.1 in the control and it was significant at 5 percent level.

Overall Acceptability

The overall acceptability of two combinations taking into account of various sensory attributes like appearance, colour, flavour, taste, consistency, it was found that in overall acceptability also probiotic beverage has secured maximum score of 4.5 and significantly different from the non probiotic (4.0).

4.4.2. Chemical analysis of the probiotic beverage

The chemical analysis of the probiotic beverage was carried out to determine the changes occurred when the product was converted to probiotic**.** The chemical constituents such as TSS, pH, titrable acidity, reducing sugars and total sugars of the probiotic beverage were analysed.

Table 17: Chemical analysis of the probiotic product

Product	TSS(^{0}brix)	pH	Acidity (%)	Reducing Sugars (g%)	Total Sugars (g%)
Probiotic beverage	**24 brix**	**6.6**	**0.083**	**35**	**86.20**
Non probiotic Beverage	23 brix	6.5	0.076	29.40	78.11
t value	-	-	14*	6.64*	8.61**

*- Significant at 5%, ($p<0.05$)

4.4.2.1 TSS

TSS of probiotic beverage was 24^0 brix and that of non probiotic beverage was 23^0 brix .

4.4.2.2pH

pH is one of the important constituent which plays an important role in the growth of microorganisms in the food. pH of Probiotic beverage was recorded as 6.6, which is slightly higher than the non probiotic beverage which is having pH of 6.5 .

4.4.2.3Acidity

Probiotic beverage recorded higher acidity of 0.083% than the non probiotic with an acidity of 0.076%. Significant difference at 5 percent level was noted in acidity. Acidity of the beverage slightly elevated due to the probiotication process.

4.4.2.4. Reducing Sugars

Reducing sugars of the two formulations was calculated and presented in the Table 17.Significant difference was recorded in the reducing sugars in the probiotic beverages and non probiotic beverages and it was found to be 35g/100g and 29.4g/100g respectively.

4.4.2.5 Total Sugars

Total sugars of the probiotic beverage enhanced and recorded total sugar content of 86.20 g of glucose/100g of juice, while non probiotic beverage recorded 78.11 g of glucose/100 g of juice. Statistical analysis also revealed significant difference at 5 percent level.

4.4.3 Nutrient analysis of the probiotic Product

Table 18: Nutrient analysis of the probiotic product

Product	Energy(kcal)	Carbohydrates(g)	Protein(g)	VitaminC(mg)
Probiotic beverage	**288**	**72.66**	0.82	**0.072**
Non probiotic beverage	258	64.66	0.47	0.063
t value	59	5.29*	35*	10.8*

***-** Significant at 5% , ($p<0.05$)

4.4.3.1 Energy

Energy content of the probiotic beverage was estimated as 288kcal/100g which was slightly higher than the non probiotic beverage of 258kcal/100g.

4.4.3.2 Carbohydrate

As mentioned earlier carbohydrate is one of the important energy yielding nutrients. Carbohydrate content of probiotic beverage was estimated as 72.66 g /100 g of sample, which was slightly higher than non probiotic beverage with a carbohydrate content of 64.66 g /100 g .Statistical analysis of the product revealed that there was significant difference at 5 percent level.

4.4.3.3 Protein

Protein content of probiotic beverage is 0.82g/100g, while non probiotic beverage is 0.47g/100 g. Protein content of the probiotic beverage is higher than that of non probiotic beverage as the probiotication process improves the availability of the protein content of the beverage though it was negligible.

4.4.3.4 Vitamin C

Vitamin C content of the beverage recorded as 0.072 mg /100 g for probiotic, while non probiotic with protein content of 0.063 mg/100g with a significant difference at 5 percent level. Statistical analysis also revealed that there was a significant difference at 5 per cent level

4.4.4 Mineral content of the probiotic product.

Table 19: Mineral content of the probiotic Beverage

Product	Iron (mg/100g)	Sodium (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Total Minerals (%)
Probiotic beverage	**0.59**	**1.5**	**0.5**	**320**	**2.0**
Non probiotic beverage	0.25	1.1	0.4	280	1.5
t value	35	-	-	39	

Significant at 5 percent $P<0.05$

4.4.4 Mineral Constituents

Iron content of the developed beverage was assessed and it was clear that iron content of 0.59 mg/ 100 g of sample which was higher than the non probiotic with an iron content of 0.25 mg/100 g. Sodium content of probiotic beverage was 1.5mg/100g while non probiotic beverage had a sodium content of 1.1. Likewise potassium content of probiotic beverage was 0.5mg/100g as against non probiotic beverage had potassium content of 0.4mg/100g. From the table it was clear that there was no difference in the sodium and potassium content of the probiotic beverage. By the probiotication process, the calcium content of the beverage increased to 320mg/100 from 280 mg /100g.Total minerals of the probiotic beverage (1.5%) was higher than non probiotic beverage (2.0%).

4.5 Shelf stability of the Probiotic beverage.

Shelf life of any product is important for market potential. Azanha and Faria (2005) reported that shelf life is the recommended time that products can be stored, during which the quality of the goods remain acceptable under specified conditions of storage. .

Product quality is often influenced by concentration of a chemical compounds and microbiological index or moisture content (Gyesley, 2003).The beverage was stored under ambient and refrigerated condition. The quality of the formulation during storage period was assessed in terms of their sensory qualities, chemical qualities such as acidity, TSS and total sugars and viable count of the *Lactobacillus acidophilus*.

4.5.1 Storage at ambient condition

Table 20: Changes in the Sensory quality of the probiotic beverage

Storage (days)	Appearance (Mean ranks)	Colour (Mean ranks)	Flavour (Mean ranks)	Taste (Mean ranks)	Consistency (Mean ranks)	Overall Acceptability (Mean ranks)
First	19.0	20.0	24.60	23.90	22.0	21.5
Second	16.0	15.35	15.0	16.50	17.0	17.0
Third	12.0	15.25	8.90	6.10	9.6	8.0
K value	6.84	6.75	22.15	6.76	11.81	16.9
C.D(0.05)	11.49					

The changes in sensory attributes of the probiotic beverage at ambient storage temperature are shown in Table 20.

The mean rank value obtained for appearance on the twenty fourth hour was 19 which was decreased to 16 on Forty eighth hour and then to 12.0 on third day. Significant decrease was recorded in the appearance attribute when stored under ambient condition. Significant colour change was recorded in the formulated beverage and rank means recorded as 20 on first day to 15.25 on the third day. Significant difference was noted in the colour during storage at ambient condition. Pleasing flavour observed in the probiotic beverage decreased drastically from 24.6 on first day to 8.9 in the third day. This decrease was highly significant. Similarly taste score of the drink stored at ambient condition decreased from 23 to 6.9. With respect to consistency cloudiness appeared in the drink and rank means significantly decreased from 22- 9.6. On the whole overall acceptability of the beverage was also found to be reduced from 21.5-8.0.

The mean ranks obtained for all parameters during storage decreased from first to third day when probiotic beverage was stored at ambient condition. So it

can be inferred that the ambient condition is not recommended for the storage of probiotic beverage.

According to a consensus made with the panelists during sensory evaluation, it was determined that the main descriptors that characterized the product were acidity and sweetness, with acidity being the attribute responsible for the sensory difference perceived by the panelists.

Table 21: Changes in chemical constituents of probiotic beverage

Days of Storage	**TSS(^{0}brix)**	**pH**	**Acidity (%)**	**Total Sugars (g%)**
First day	23.0	6.5	0.073	57.7
Second day	24.3	6.4	0.251	39.0
Third day	26.0	6.3	0.323	30.1
C.D (0.05)	0.666	-	0.006	2.515

The changes in chemical constituents of the probiotic beverage stored at ambient condition are shown in table 21.

The TSS of the probiotic beverage slightly increased from 23- 26.0 from first to third day of storage at ambient temperature. It may be due to the increase in the rate of fermentation. The increase was found significant at 5 percent level. Likewise acidity of the probiotic beverage increased considerably from 0.07% - 0.32%.Significant difference in the acidity was noted in the beverage at 5 percent level. pH of the probiotic beverage decreased from 6.5 – 6.3 from first to third day. The total sugar content of the probiotic beverage also decreased during ambient storage from 57.7 – 30.12 g of glucose/100 g.

Table 22: Viable count of *Lactobacillus acidophilus* in the probiotic beverage during storage in the ambient condition

Storage (h)	Count (x 10^9cfu/ml)
24h	59.4
48h	33.0
72h	21.4

The viable count of the probiotic beverage was assessed during storage at the ambient condition and showed a tremendous decrease in the viable count. The initial viable count was 74.4 x 10^9 cfu/ml, after 24 hours it decreased to 59.4 x 10^9 cfu/ml, after 48 hours it decreased to 33x 10^9 cfu/ml and after 72 hours, it decreased to 21.4x 10^9 cfu/ml.

Chemical analysis was discontinued on third day as there was a tremendous change in the beverage with respect to sensory attributes. Results clearly depicted the beverage was not fit to consume if stored at ambient condition. It can be inferred that the ambient condition is not proper for the shelf life of the probiotic beverage as it affects the sensory qualities, chemical constituents and viable count in 72 hours.

4.5.2 Storage at refrigerated Condition

Table 23: Changes in sensory qualities of the probiotic beverage

Storage period (d)	Appearance	Colour	Flavour	Taste	Consistency	Overall acceptability
Second	48.5	49.0	50.5	54.0	47.5	49.0
Fourth	48.5	49.0	50.5	54.0	47.5	49.0
Sixth	48.5	49.0	50.5	54.0	47.5	49.0
Eighth	48.5	49.0	50.5	54.0	47.5	49.0
Tenth	26.1	22.6	31.9	27.7	24.7	22.6
Twelvth	17.1	17.9	14.5	19.5	20.3	17.9
Fourteenth	11.3	11.9	7.9	7.7	13.4	11.9

Fifteenth	11.2	11.5	7.2	6.8	12.3	11.5
K value	56.1*	57.93 *	58.47*	58.9*	48.87*	57.93*
C.D(0.05)	19.69					

Sensory analysis of the probiotic beverage stored in refrigerated condition was also carried out. It was clear from the table that till the eighth day of storage in refrigerated condition, all the sensory attributes remained constant. After the eighth day, mean rank value obtained for appearance was decreased from 26.1- 11.2, Mean rank obtained for colour decreased from 22.6-11.5 from tenth to fifteenth day, like wise mean rank value of the flavour decreased from 31.9-7.2. Rank value for taste also decreased from tenth to fifteen day by 27.7-6.8, consistency also decreased with the other sensory attributes from 24.7-12.3 by fifteenth day. It affects the rank means for overall acceptability also decreased with from 22.6-11.5 by fifteenth day. After fifteenth day it was not at all acceptable to the panelists.

Table 24: Changes in chemical constituents of probiotic beverage

Days of Storage	**TSS(^{0} brix)**	**pH**	**Acidity(%)**	**Total sugars(g%)**
Second Day	24.0	6.5	0.073	40.10
Fourth Day	27.0	6.5	0.074	28.73
Sixth Day	27.0	6.5	0.083	28.63
Eighth Day	27.0	6.4	0.093	27.07
Tenth Day	27.0	6.3	0.215	27.17
Thirteenth day	28.0	6.3	0.222	26.21
Fourteenth day	31.0	6.2	0.233	25.77
Fifteenth day	31.0	6.2	0.233	25.77
C.D (0.05)	0.855	0.035	0.430	0.308

The above table shows the results of the chemical constituents during the storage of probiotic beverage in refrigerated condition. There was a slight change in the three chemical constituents such as TSS, acidity and total sugars.

TSS of the probiotic beverage during storage increased from 24^0 brix to 31^0 brix. pH of the beverage remained stable till 8 days after which it showed declining trend. Acidity ranged from 0.07 to 0.23 during storage of 15 days at refrigerated condition .With respect to acidity, a significant increase was recorded and was significant at 5 per cent level. The total sugar content of beverage at storage in refrigerated condition decreased from 40.0 - 25 g of glucose/100g.

Table 25: Changes in viable count of *Lactobacillus acidophilus*

Days of storage	**Count ($x10^9$cfu/ml)**
Second	21.2
Fourth	42.4
sixth	33.6
Eighth	38.6
Tenth	54.4
Twelveth	76.8
Fourteenth	94.8
Fifteenth	94.8

The above table shows the viable count of the probiotic beverage during its storage in the refrigerated condition. From the table it is clear that the viable count increased till fourth day of storage from $21.2x10^9$ cfu/ml to $42.4 x10^9$ cfu/ml. Decline in the viable count was noticed from 6^{th} and 8^{th} day $33.6 x 10^9$ cfu/ml and there after an increased in the viable count was recorded. Maximum count was observed by 15^{th} day of refrigerated storage ($94.8 x 10^9$ cfu/ml). So it can be concluded that the beverage was having shelf life of 10 days at refrigerated condition with consumer acceptance with desirable count.

4.6 The Consumer acceptance of the probiotic beverage

Consumer awareness and preference decides the success of food products standardized. Consumers use numerous products criteria to evaluate whether a food product satisfies their expectations and requirements (Gellynck *et al*., 2008).

Consumer acceptance of the probiotic beverage was carried out using the hedonic rating scale. Consumer acceptance was done among 30 subjects comprising of 10 school students (11-14 years), 10 college students and 10 professionals. The results of the hedonic rating are presented below:

Table 26: Consumer acceptance of the probiotic beverage

Particulars	**Consumer acceptance in percentage**		
	Scores	Probiotic beverage	Non probiotic beverage
Like extremely	9	30 (9)	23.33 (7)
Like very much	8	60 (18)	46.66 (14)
Like moderately	7	10 (3)	6.66 (2)
Like slightly	6		23.33 (7)
Neither like nor dislike	5		
Dislike slightly	4		
Dislike moderately	3		
Dislike very much	2		
Dislike extremely	1		

Consumers revealed their preference for the products and found that 90 percent recorded 'likes extremely' to 'likes very much' for probiotic beverage as against 69 percent for non probiotic beverage.

4.7 Yield ratio

Yield ratio of the probiotic beverage was assessed. In order to find out the processing loss and to ensure market potential. Yield ratio of the beverage was obtained as 0.83. There was not much processing loss in the formulation of the beverage. Processing loss was found to be minimum with aloevera pulp preparation.

The results clearly indicated that the beverage developed was good and in comparison with non probiotic beverage.

4.8 Cost of the probiotic beverage.

Cost of the beverage was worked out using cost of the ingredients used including the cost of the culture procured along with 10% overhead charges accounting fuel, electricity, labour etc. It was found that the cost of formulated beverage was Rs 15/- for 100 ml. The cost of the formulated beverage was lesser than the commercially available probiotic beverages.

So it can be concluded that the formulated new probiotic beverage was outstanding in all the qualities parameters viz, sensory, chemical and nutritional and found economically viable.

DISCUSSIONS

5. DISCUSSION

The results of the present study entitled "Development and Quality evaluation of probiotic honey beverage "are discussed under the following headings:

5.1) Product formulation and standardization
5.2) Quality assessment of the beverage.
5.3) Probiotication process of the beverage.
5.4) Quality assessment of the probiotic beverage.
5.5) Shelf stability of the probiotic beverage.
5.6) Consumer acceptance of the probiotic beverage.
5.7) Cost and yield ratio of the probiotic beverage.

5.1 Product formulation and standardisation

Standardisation plays a key role in product formulation which facilitates the growth of food industry as it is a pre requisite of any food based industry. According to Liaqt *et al* (2009) recipe standardization is important to achieve optimal accuracy in determining the nutrient composition. Product diversification is the present need due to rapid changes in socio economic and living styles (Singh and Gopalakrishnan, 2002). Different types of beverages cater to the nutritional requirements of the young, adult and old age population as it is readily available and easy to consume.

5.1.1 Standardisation of beverage.

Various steps involved in the standardisation procedure followed in the present investigation are preliminary processing, optimization of ingredients, blending, formulation and quality evaluation.

According to Bhagwan and Awadhesh (2014) the blending technology has become an important tool in modern beverage processing which enable to formulate beverages of superior quality with sensory, nutritional and medicinal properties of two or more plant species. Carvalho *et al*. (2007) opined that

juice blending is one of the best methods to improve the nutritional quality of a beverage. It can improve the vitamin and mineral content depending on the kind and quality of ingredients used.

5.1.1. Optimization of ingredients

In the present study, the product was standardized by blending the different proportions of ingredients in different combinations and dilution with water to attain proper consistency and taste. The intention was to incorporate honey, aloevera pulp and soymilk in an ideal proportion to obtain highest sensory scores and appeal. This was achieved through 'trial and error method'. Chou and Hou (2000) reported that soymilk is highly suitable for the growth of the lactic acid bacteria especially *bifidobacteria and lactobacillus.*. Wang *et al*. (2002) reported that production and use of fermented soymilk drinks as probiotic, mainly soyabean yogurt, can be supplemented with oligo fructose and inulin due to its protein quality. Honey is symbiotic (combination of prebiotic and probiotic) which is a suitable ingredient for the effective growth of lactic acid bacteria (Sampath *et al*., 2010).

Honey, aloevera pulp and soy milk were blended in nine different proportions and evaluated for sensory attributes. Out of the nine combinations tried out, three combinations were adjudged to be superior based on the rank means obtained for sensory qualities. The mean ranks for overall acceptability of three combinations C_9, C_8 and C_6 were 69.5, 66.0 and 59.0.

The proportion of honey, aloevera pulp, soymilk and water in the C_9 combination was 25: 10 : 25 : 40 . The proportions of honey, aloevera pulp, soymilk, water in the C_8 was 30: 10 : 20 : 40 while C_6 was having honey, aloevera pulp, soy milk and water in the proportion 40 : 10 : 15 : 35.

The best combination selected was the one having honey and soya milk in equal amount with 10 percent aloevera pulp. The other two combinations namely C_6 and C_8 were with blend of honey and soymilk in the ratio 4.0: 1.5: and 3.0: 2.0 with 10 percent aloevera pulp. When the proportion of aloevera was enhanced,

the taste of the combination was found to decrease as the bitterness dominated in the beverage. Deen and Tiwari (2014) standardized a RTS beverage with best blend containing 75 percent bael pulp and 25 percent aloevera gel.

In a study conducted by Deka (2000) suggested that the blending of mango, lime, aonla, grape and pineapple pulp/juice in appropriate proportion could improve the quality of the RTS beverages. Mandal (2003) reported that blend consisting of 75 per cent pineapple juice and 25 per cent phalsa juice was used for the preparation of blended RTS. Karanjalker *et al*. (2013) found that recipe with 70 percent guava nectar and 30 percent soymilk has attained highest sensory scores. Bhagwan and Awadesh (2014) developed RTS beverage with the best blend of 90 percent mango and 10 percent ginger juice..

5.1.2 Sensory evaluation of the formulated beverages

Organoleptic evaluation has been defined as a scientific method used to evoke, measure analyse and interpret those responses to products as perceived through the senses of sight, smell, touch and taste (Stonel and Sidel, 2002). Bhagwan and Awadhesh (2014) opined that attractive appearance, appealing flavour, nutrient retention, medicinal value and other organoleptic qualities are the main consideration in the standardization of different ratios of blend components which meets the consumers preference and improves the marketability of the new blended p In the present study in sensory evaluation, C_9 was adjudged to be the best and has got higher acceptance among the panelists. The mean ranks obtained for the best combination (C_9) for various sensory attributes such as appearance, colour, flavour, taste, consistency and overall acceptability were 67.6, 69.0, 69.2, 72.9, 75.0 and 69.5 respectively. The mean ranks obtained for sensory attributes of the combination C_8 were 59.2, 58.8, 62.0, 70.1, 68,5 and 66.0; while that of C_6 combination were 55.0, 55.2, 58.4, 61.5, 58.4 and 59.0 (Fig1). Sindhumati and Premalatha (2013) developed RTS with pineapple and papaya in the ratio 50:50 which has got higher sensory

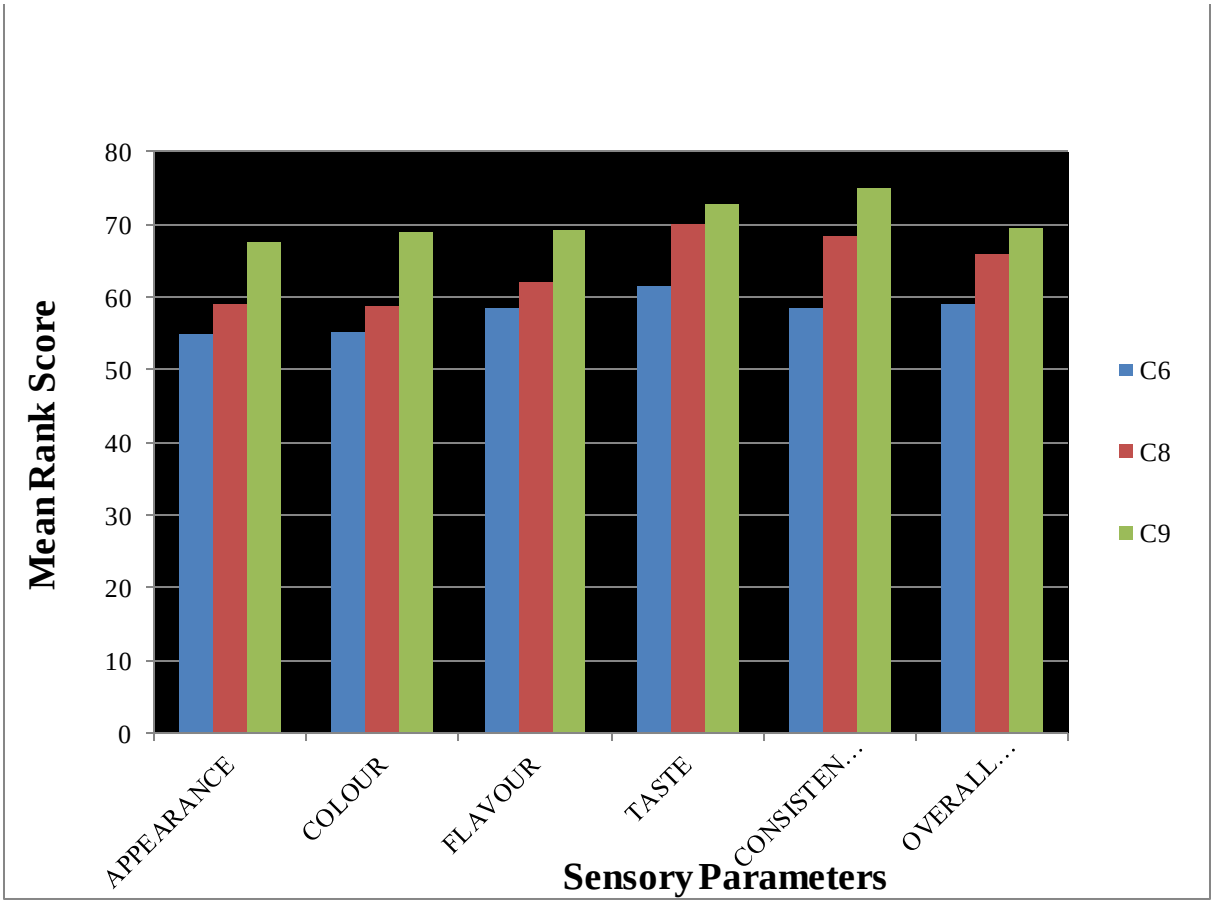


Fig 1. Mean rank scores for sensory attributes of selected combinations

appeal with the scores 9.0, 9.0, 9.0, 9.0 and 9.0 for colour, appearance, taste, flavour and overall acceptability.

The mean ranks obtained for appearance of the selected three combinations ranged from 67.6-55.0. Significant difference was observed in the appearance of the beverage in the three combinations. Dhamsaniya and Varshney (2013) reported that mean score obtained for appearance of the whey based RTS from banana ranged from 5.50-6.58 in hedonic rating. Boghani *et al*. (2012) reported that appearance of the blended papaya- aloevera RTS beverage enhanced with increase in the concentration of *aloe vera* juice up to a level of 10 percent while further increase in *aloe vera* juice content reduced the appearance profile.

The mean ranks obtained for colour of the selected three combinations ranged from 69.0-55.2. Significant difference was indicated in the colour of the beverage at 5 percent level. All the three combinations were found to have pale cream to cream colour. Desale *et al*. (2011) reported that mean scores obtained for colour and appearance on hedonic rating were ranged from 8.02- 8.70 in whey chhana beverage.

The mean ranks obtained for flavour of the combinations ranged from 69.2-58.4 with a significant difference at 5 percent level. Dhamsaniya and Varshney (2013) reported that mean score obtained for the flavour of whey based RTS from banana ranged from 3.58-6.83. Desale *et al*. (2011) reported that mean scores obtained for flavour of whey chhana beverage on hedonic rating ranged from 8.03- 8.62.

With respect to taste, the mean ranks secured for taste in the combinations ranged from 72.9- 61.5 with a significant difference at 5 percent level. It was observed in the present investigation, all the three proportions which secured higher rank means were the ones which incorporated 10 percent aloevera pulp. Taste of the beverage varied with the percentage of aloevera pulp. Soymilk when

incorporated at 15, 20, and 25 percent imparted good taste, while honey gave sweetness and colour to the beverage.

Drewnowski and Carmen (2000) reported that bitterness was the characteristic that determined the preference of consumers for a product. Bitter sensation is not by itself appealing to most people, which could be the reason for low acceptability of bitter gourd juice (Satkar *et al*., 2013). It was also noted in the study that colour of the beverage increased with the increase in honey and while decreased with addition of soymilk.

Boghani *et al*. (2012) prepared RTS with papaya and aloe vera juice in different ratio and found that the sample with 5 and 10 percent of aloevera juice reached the highest hedonic scores.

The mean ranks for consistency of the three combinations ranged from 75.0- 58.4. Dilution of the beverage with water gave proper consistency, and fluidity to the beverage. The turbidity/ consistency of the developed combinations varied with the amount of soymilk incorporation and water used for the formulation. Variation in the consistency of formulations was due to the variation in the density of the ingredients used. Sivasankar, (2013) noted that slight turbidity or cloudiness in orange juice was acceptable to the consumers while it was not acceptable in apple juice.

Dhamsaniya and Varshney (2013) reported that whey based ripe banana RTS beverage with 15 percent banana juice, 3 percent M. arvenis extract, 8g sugar powder and 77 ml milk per 100 ml of RTS beverage found to be superior.

On the whole, the overall acceptability of the three combinations ranged between 69.5-59.0. Rank means for overall acceptability for C_9, C_8 and C_6 were 69.5, 66.0 and 59.0. C_9 combination was having honey, Aloevera pulp, soymilk and water in the proportion 2.5: 1.0: 2.5: 4.0. Dhamsaniya and Varshney (2013) reported that mean score obtained for overall acceptability of the whey based RTS from banana ranged from 3.50-7.33.

5.1.3 Flavour enhancement in the selected combinations

Flavour means an overall integrated perception of taste and aroma associated with the product (Meilgaard *et al*., 2007). Flavour of the beverage is of prime importance in the acceptance or rejection of a product. The best three combinations obtained were subjected to flavour enhancement treatment by the addition of four different flavours namely lime emulsion, chocolate, vanilla and cardamom. The mean ranks obtained for overall acceptability of flavours added to the C_8 combination were 16.6, 15.3, 16.7 and 13.7, while mean ranks for the flavours for C_6 were 20.1, 11.1, 10.7 and 12.1. The overall acceptability scores of flavour of the beverage (C_9) were 9.8, 19.1, 20.1 and 20.7 respectively for lime, chocolate, vanilla and cardamom respectively. Results revealed that in all the three combinations of beverage with four flavours, cardamom was found to be superior

Sindhumathi (2002) reported that the flavoured (ginger + cardamom) papaya blended RTS was more acceptable than the plain papaya RTS beverage. Joshi *et al*. (2003) reported that the organoleptic quality of RTS beverages prepared from fruits such as plum and watermelon could be increased by the addition of spice extracts of ginger, black pepper, mint, cardamom and cumin. These flavours, apart from their appetizing properties also possess medicinal and therapeutic values, which have a profound effect on human health, since they affect many functional processes. In the present study, formulated beverage was flavoured with cardamom which subsequently enhanced consumer appeal.

5.2 Quality assessment of the beverages

The quality of the food is the important factor for the acceptance and marketing of a new innovative product. These include organoleptic qualities, nutritional value, microbiological safety, cost and convenience.

Nambiar and Parnami (2008) reported that development of nutritious and organoleptically acceptable recipes with locally available food is a challenge for the food scientist and the benefit food- based strategies to prevent micronutrient

malnutrition. In the present investigation, selected combinations with different proportions of honey, aloevera pulp and soymilk were further ascertained for sensory, chemical and nutritional composition

5.2.1 Chemical analysis of the formulated beverages

Saxsena (2003) opined that laboratory analysis is one of the best methods to assess the quality of different constituents present in the products. In the present investigation, the chemical constituents such as TSS, pH, titrable acidity, reducing sugars and total sugars of the selected combinations were ascertained.

Generally different beverages will have different TSS. TSS is an important chemical constituent of the RTS beverages. TSS offer taste to the product. TSS of the three selected combinations was in the range of 20^0-23^0 brix. TSS of the formulated beverage varied depending upon the proportion of honey, soyamilk and aloevera pulp used while blending. In the formulation of the beverage, honey alone was used as a sweetener and it is a pure source with added therapeutic potency. FSSAI recommended TSS of RTS as minimum 10 percent.. TSS of formulated beverage was higher than the recommended TSS for RTS. The beverage has additional benefit since no sugar was added to the beverage. Downey *et al*.(2005) found that in most honeys, fructose predominates and tends to make the taste slightly sweeter than sugar.

Balaji and Prasad (2014) standardized kinnow – anola RTS beverage with TSS of 15.01^0 brix. Deen and Tiwari (2014) formulated RTS with TSS 12.0^0 brix. Chauhan *et al* . (2012) developed herbal RTS beverage having TSS of 15.24^0 brix. In all such beverage cane sugar was used as sweetener.

pH and acidity are important chemical factors for consumer acceptance of the RTS beverages. pH of the three formulations ranged from 4.4-6.5 while acidity of the formulations ranged from 0.065- 0.08%. With the increase in the pH, the acidity was decreased. pH of the formulated beverages increased with the addition of soymilk in a proportion from 15 to 25 percent. Divya and Archana(2009)

formulated whey guava beverage with pH 3.83-4.20. Singh *et al*. (2014) reported that pH of whey guava beverage ranged from 3.39-4.15. pH and acidity was influenced by the content of the ingredients used. In the formulated beverages contain only natural ingredients and no preservative were added to maintain pH and acidity.

Amaravathi *et al*. (2014) formulated spiced pineapple RTS with TSS 15^{o} Brix, pH 3.9 and acidity 0.25 percent as per FSSAI specification. Deen and Tiwari(2014) standardized bael and Aloevera RTS beverage with acidity 0.25 percent.

Bhardwaj *et al*. (2005) opined that increase in pH can be due to decrease in titrable acidity which affects the organoleptic quality of juice. Singh *et al*. (2014) reported that acidity of whey guava beverage standardised varied from 1.24- 1.49%.

The consumer acceptance as well as the taste profile of the RTS beverage found to increase with the sugar content of the beverage. In the current investigation it was found that reducing sugars of the combinations ranged from 26-32 gram percent and total sugars ranged from 73g%-93g%. Reducing sugars and total sugars found to decrease with quantity of honey and increased with addition of soymilk.

Reducing sugars and totals sugars of formulated bael and aloevera RTS were reported as 2.30 per cent and 10.21 per cent respectively. (Deen and Tiwari, 2014). Spiced pineapple RTS formulated by Amaravathi *et al*. (2014) was found to have reducing sugars and total sugar of 4.20percent and 13.61%. Singh *et al*.(2014) standardized whey guava beverage with reducing sugar ranged from 5.068- 5.88g/100g,while total sugars ranged from 24.32- 24.85g/100g.

Deka *et al*. (2005) also formulated processed mango pineapple spiced RTS beverage with TSS 10^{0}Brix, acidity 0.23 per cent, reducing sugar 4.22 per cent, total sugar 9.58 per cent.

5.2.2 Nutrient analysis of the selected combinations.

Kalia and Sood (1996) defined nutritional quality as the combination of chemicals that has significance in determining the degree of acceptability of the product to a user, based on its quality and sensory attributes. Hence nutritional quality assessment of the selected beverages was carried out with respect to nutrients viz., energy, carbohydrate, protein, vitamin C and minerals.

Calorie content of the beverages formulated were found to be in the range of 296-256kcal per 100 ml and was significantly different at 5 percent. Generally beverages yield more calories as they are source of quick energy. Shruti (2005) reported that the energy content of malted health drink mix and spiced health drink mix as 318and 314 kcal/100g respectively.

Carbohydrate is one of the energy yielding nutrients and its quantity in honey is very high. Carbohydrate content of the beverage ranged from 64-81g percent and it was mainly contributed from honey as the selected combinations contain 25- 40 % honey in the formulations. Protein content was negligible (0.4- 0.8%) and vitamin C content of the beverage was very low (0.04-0.08 mg %). Ingredients used for the formulation do not contribute much to the beverage with respect to protein and vitamin C. Singh *et al*. (2014) reported that protein content of whey guava beverage ranged from 0.293-0.344. Whey proteins are of higher value than other animal proteins (Devaraj, 2005).

In the case of minerals, a wide difference was observed in the case of iron and calcium in the formulated beverages and was significant at 5 percent level. Sodium content of C_9, (1.5mg/100g), C_8 (1.1 mg/100 g) and C_6 (2.3 mg/100 g) respectively. Potassium content of C_9, C_8, C_6 were 0.4mg/100g, 0.5mg/100g and 0.8 mg/100g respectively.

It can be concluded that, three selected combinations varied in their sensory, chemical and nutritional parameters. Various constituents in the beverage are designed to suit and support the probiotication process. From the sensory analysis it

was found that the C_9 is best in all the sensory attributes with cardamom flavour. Besides, C_9 was recorded highest TSS and pH, while the other three chemical constituents (acidity, reducing sugars and total sugars) were higher for C_6 combination which was having higher amount of honey. Likewise C_6 combination was having higher content of macronutrients such as energy and carbohydrates.

5.3 Probiotication process of beverage

Relationship between certain foods and health benefits and development of foods that promote health and well being is one of the key research priorities of food industry (Yoon *et al*., 2004). This trend has favored consumption of functional foods that enriched with physiologically active components such as prebiotics, probiotics, vitamins, minerals, dietary fiber, plant sterol and other functional ingredients (Betoret *et al*.,2011).

Marchand and Vandenplas (2000) reported that one way of creating a functional food is by inclusion of ingredients such as probiotics and prebiotics to levels that enable the consumer to derive optimal health benefits.

Probiotication is one of the methods used to produce fermented functional foods with live microorganisms. (Saarela *et al*. 2002 ; Rafter, 2003) reported that addition of probiotics to food provides several health benefits including reduction in the level of serum cholesterol, improvement of gastrointestinal functions, enhancement of immune system and reduction in risk of colon cancer The beneficial effects of food with added live microbes (probiotics) on human health are being increasingly promoted by health professionals.

Molin (2001) formulated oatmeal fruit probiotic drink with *Lb. plantarum* with viable count of 10^{12} cfu/ml. Yoon *et al*. (2004) studied the suitability of tomato juice as a raw material for production of probiotic juice by lactic acid bacteria and found that the product could serve as a health beverage for vegetarians and consumers who are allergic to dairy products. Angelov *et al*. (2006) formulated a symbiotic functional beverage from the oats by combining *L. plantarum* and whole

grain oat substrate. Rakin *et al*. (2007) developed beetroot and carrot probiotic juice with *L. acidophilus*.

Selection of culture, optimization of dosage and pH, optimization of incubation period and pretreatments prior to probiotication are essential steps in the formulation of a probiotic beverage.

5.3.1 Selection of culture

Based on existing standards and from a health view-point, it is very important that probiotic strains retain their viability and functional activity throughout the shelf life of product. Nadal *et al*. (2010) reported that presence of plant-based ingredients may improve the growth of probiotic cultures in milk such as tomato juice, peanut milk, soy milk, carrot and cabbage juice.

The probiotic microorganisms consist mostly of strains of the genera *Lactobacillus*, but not exclusively. These bacteria have been used widely in dairy and non dairy products (Holzapfel and Schillinger, 2002).

In the present study, *Lactobacillus acidophilus* was the organism used for the probiotication process. Ng *et al*. (2009) reported that *Lactobacillus acidophilus* is a well known and well studied probiotic microorganism. Martensson *et a*l. (2002) reported that probiotic culture *L. acidophilus* grows well in non dairy based probiotic foods.

Cruce and Goulet, (2001) reported that *Lactobacilli acidophilus* were added to a variety of dairy-based products such as fermented milks and yogurts for their probiotic human health benefits. The specific medium required for the growth of *Lactobacillus acidophilus* is MRS (De Mangosa Rosa Sharpe).

Hoppe and Larsen, (2008) reported that *Lactobacillus acidophilus* has no adverse effects on the taste, appearance or palatability of the product. It is able to survive in the product until consumption. Sweet acidophilus milk is an example of a sweet milk product with added probiotic *L. acidophilus* (Mattila-Sandholm *et al*., 2002).

5.3.2 Optimization of pH

pH is an important character in the probiotication process and pH varies with the culture used for the process. After the selection of the culture, optimization of pH for the growth of organism was finalized. Culture was inoculated at varying pH from 4.0- 7.0 and found that growth was adequate with a pH from 6.2- 6.6. In the present study, since the culture depicted good growth at a pH 6.5, the combination C_9 was finally selected and the pH was optimized at 6.5.

C_8 combination with pH 5.5 was discarded, and further process was proceeded with C_9 combination.

Shukla *et al.* (2013) developed a probiotic beverage based on whey and pineapple juice with *Lactobacillus acidophilus* with a pH of 4.82. In another study, Kockova and Valik (2014) formulated pseudocereal based probiotic food with *Lactobacillus rhamnosus GG* and at pH 5.66.

5.3.3 Optimization of dosage of inoculum, temperature and incubation period

The dosage of culture and incubation period of the inoculum is another important criterion in the probiotication process.

Three doses viz., 1.0 1.5 and 2.0 per cent inoculum was inoculated and viable count was recorded at varying time interval of 3 hours, 6 hours and 9 hours. The maximum viable count obtained with 1 per cent inoculum was 85. 8 x 10^9 cfu/ml, 1.5 per cent inoculum attained viable count of 115 x 10^9 cfu/ml and 2.0 per cent inoculum depicted maximum viable count of 135x 10^9 cfu/ml at 6 hours of incubation. The viable count of *Lactobacillus acidophilus* (1.0 per cent) in beverage at 3hours, 6 hours and 9 hours of incubation were 46x 10^9 cfu/ml, 85.8 x 10^9 cfu/ml and 64.4 x 10^9 cfu/ml respectively. In the present study the dosage was optimized to 1 percent since the viable count obtained at 6 hours of incubation itself attained the recommended level of 10^6- 10^9 cfu/ml (Fig2). Zeynab *et al*. (2010) incorporated 1.0 per cent *Lactobacillus acidophilus* for development of functional symbiotic acidophilus milk.

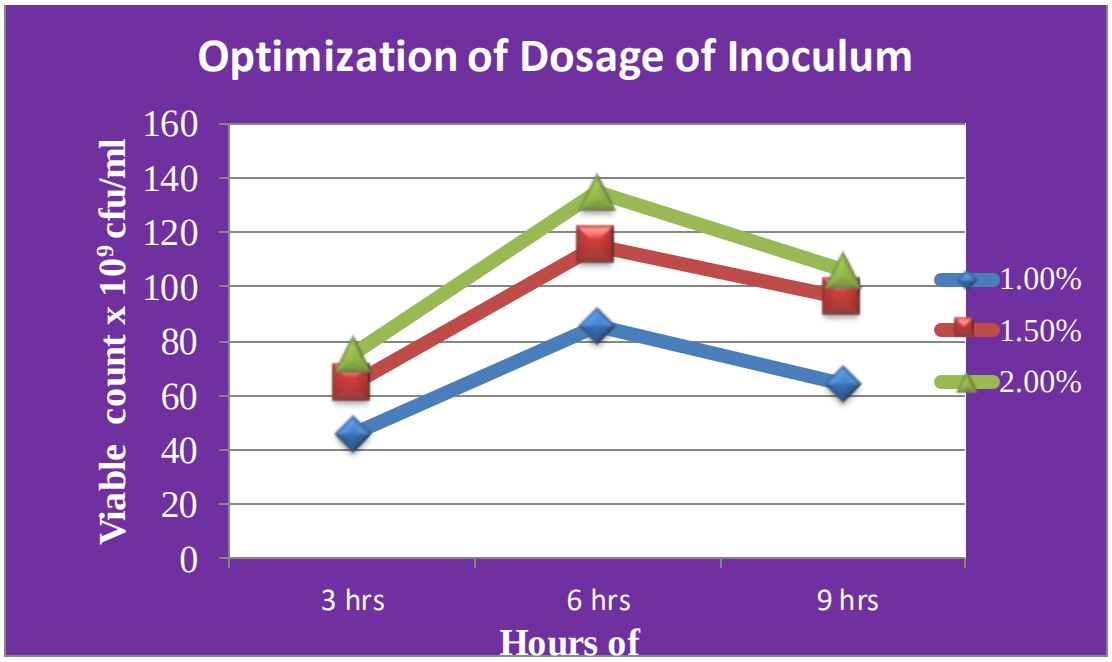


Fig 2: Optimization of dosage of inoculum

Santos (2001) formulated probiotic cassava beverage with 8% inoculum of *L. plantarum* with incubation period and temperature as 16 hours and 35^0C with viable count of 2.3 x 10^9 cfu/ml. Angelov *et al*. (2006) reported that *L. plantarum* in probiotic tomato juice attained maximum viable count of 7.5 x 10^{10} cfu/ml at 8 hours of incubation. Shukla *et al*. (2013) standardized whey and pineapple based probiotic beverage with 1% inoculum of *Lactobacillus acidophilus* with a viable count of 4.71 x 10^7 cfu/ml at incubation period of 10 hours. Anita *et al*. (2014) formulated probiotic mixed fruit beverage with 2% inoculum of *Lactobacillus acidophilus* to attain maximum desirable count (2.1 x 10^9 cfu/ml).

In the present study, incubation temperature was maintained at 37^0 C. Shamala *et al*. (2000) reported that the optimum temperature for the growth of *L. fermentum* was 30^0 C.Pereira *et al*. (2010) reported that optimum temperature for the growth of *Lactobacillus casei* in probiotic cashew apple juice was 30^0 C and 16 hours of fermentation.

5.3.4 Pretreatments prior to probiotication

Suvarna and Bobby (2005) reported that a good probiotic agent needs to be non-pathogenic, non toxic, resistant to gastric acid, adhere to gut epithelial tissue and produce antibacterial substance. Sterilized products can retard the growth of any pathogenic organism and improves its quality. Standardized probiotic beverage should be consumer safe for market potential.

Hence selected standardised beverage was subjected to sterilization at 100^0 C and 80^0C. The viable count obtained for sterilized (100^0C) and inoculated beverage at different time intervals of 3, 6 and 9 hours were 33x 10^9 cfu/ml, 74.4 x 10^9 cfu/ml and 54.4 x 10^9 cfu/ml respectively. However sensory parameters mainly colour of the beverage altered and become intense besides curdling occurred in the beverage. Sterilization at 100^0 C was not adviced.

Therefore sterilization at 80^0C was tried. When the beverage was sterilized at 80^0 C, colour change and appeal was also found to be less. Thus it was decided to sterilize individual ingredients separately before blending except honey. Hebbar *et al*. (2003) reported that heat treatment on honey impairs its quality mainly by colour change and then antimicrobial properties.

Finally the beverage was formulated using honey, soy milk and blended with sterilized Aloevera pulp and water. Enumeration of viable count of *Lactobacillus acidophilus* at 6 hours of incubation indicated 95.8x 10^9 cfu/ml which was more than enough for desired health benefits.

Results revealed that viable count obtained from different temperatures, viability of the organism is affected and drastic decrease in viable count with increasing temperature was found. So the best method to make the formulated beverage consumer safe was sterilization of individual ingredients and then blending.

5.4 Quality assessment of probiotic beverage

Quality assessment of the probiotic beverage was essential to determine the acceptance and its quality among the consumers. Quality assessment of the probiotic beverage was carried with respect to their sensory, chemical and nutritional qualities.

5.4.1 Sensory assessment of the probiotic beverage

Sensory assessment of the probiotic beverage was carried out by keeping non probiotic beverage as control. Mean scores of ten judges was used for sensory assessment of probiotic beverage.

Mean score for appearance of probiotic beverage was 4.6 and was significantly different from non probiotic beverage and beverage becomes thicker as a result of probiotication. Mean score was taken after 6 hour of incubation.

With respect to colour attribute probiotic beverage scored 4. 7 out of 5 and was significantly higher than the control. Colour becomes more intense nd pleasing. Shukla *et al*. (2013) reported that mean score of appearance and colour of

whey and pineapple juice probiotic beverage ranges from 8.92-6.53. Junaid *et al*. (2013) reported that colour difference among the flavoured acidophilus milk with control sample was very slight.

Similarly mean score for flavour, taste and consistency secured by the probiotic honey beverage was 4.6, 4.5 and 4.7 respectively which was significantly higher than the non probiotic beverage. Shukla *et al*. (2013) reported that the mean score for flavour of the whey pineapple beverage ranged from 8.58-4.34; and consistency ranged from 8.69- 6.04 on hedonic scale. Flavour and taste of the product was found to be enhanced due to probiotication. This may be due to the process of fermentation occurred in the beverage. Reddy *et al*. (2014) reported presence of off flavours in formulated probiotic tomato and watermelon juice.

Shukla *et al*. (2013) found that mean score for flavor of whey and pineapple juice based decreased significantly with increasing fermentation time.

Overall acceptance was enhanced in the probiotic beverage when compared to the non probiotic and with a mean score of 4.5 out of 5 (Fig3).

Shukla *et al*. (2013) reported that the score obtained for overall acceptability of the whey based probiotic beverage ranged from 8.87-4.99 in hedonic rating. Highest score for overall acceptability was recorded in case of whey-pineapple juice blend fermented for 5 hours (Shukla *et al*., 2013). The mean scores obtained for developed honey based non probiotic beverage for every sensory attributes namely appearance, colour, flavour, taste; consistency and overall acceptability were 4.1, 4.1, 4.1, 4.0, 4.1 and 4.0.

According to Luckow and Delahunty (2004), the sensory characteristic of probiotic black currant juice was perfumery in odor and sour and savory in flavor.

5.4.2 Chemical analysis of the probiotic beverage.

Chemical assessment of the probiotic beverage was also carried out with non probiotic beverage as control. The chemical constituents analysed were TSS, pH, acidity, reducing sugars and total sugars.

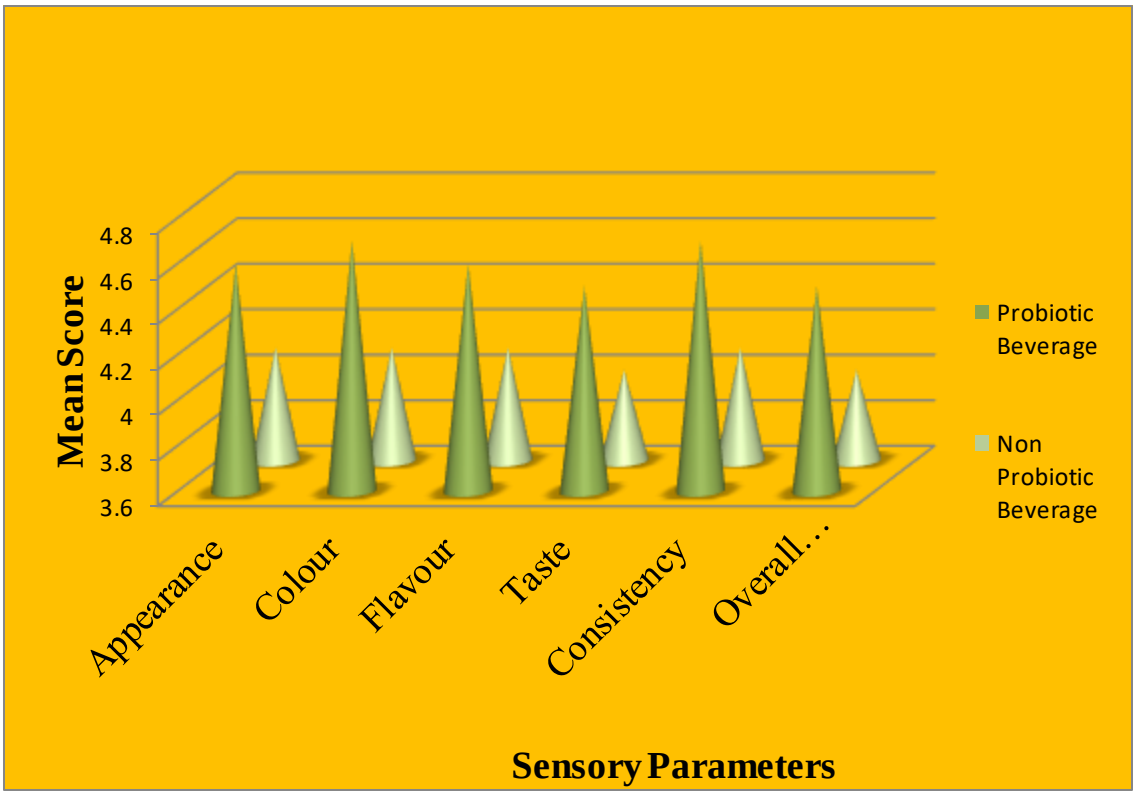


Fig 3. Sensory evaluation of probiotic v/s Non probiotic beverage

TSS of probiotic beverage was 24^0 brix and that of non probiotic beverage was with a TSS of 23^0 brix. Shukla *et al.* (2013) reported that whey pineapple juice probiotic beverage with TSS of 12.2^0 brix. pH of probiotic beverage was 6.6, which was slightly higher than the non probiotic beverage which was having pH of 6.5. Shukla *et al.* (2013) found out that higher viable counts during the initial period of fermentation resulted in comparative lowering of pH for whey fermented along with pineapple juice.

Probiotic beverage recorded higher acidity of 0.083% than the non probiotic with an acidity of 0.076%. Shukla *et al.* (2013) found that mean values of pH and acidity of whey and pineapple juice probiotic beverage were 4.36- 3.87 and 0.92- 0.54% respectively. Titrable acidity increased significantly with increasing fermentation time irrespective of the medium (Shukla *et al.*, 2013). Daneshi *et al*. (2013) found that pH and acidity of milk carrot juice drink ranged from 5.33- 6.6 and 0.13- 0.31%.

Likewise probiotic beverage recorded higher reducing sugar content of 35g/100g while probiotic with reducing sugar content of 29g/100g. Total sugars of the probiotic beverage enhanced and recorded total sugar content of 86.20 g of glucose/100g of juice, while non probiotic beverage recorded 78.11 g of glucose/100 g of juice. Bhagwan and Awdhesh (2014) reported that increase in total sugars and reducing sugars corresponded with decrease in non-reducing sugar content could be as a result of inversion of non-reducing sugar into reducing sugars. Similar observations on changes in sugars content were reported in blended RTS of phalsa and pineapple (Mandal, 2003) and karonda squash (Deen and Singh, 2012). It may infer that chemical constituents were found to enhance by the probiotication process.

Shukla *et al.* (2013) observed that whey-pineapple blend having a pH of 4.36 and titratable acidity of 0.546% gave the best flavour profile to the probiotic beverage. Sahota *et al.* (2008) formulated functional probiotic beverage 'kanji' with

TSS 4.0^0 brix, pH 3.0, acidity of 0.5%, reducing sugars of 0.95% and total sugars of 1.40%.

5.4.3 Nutritional analysis of the probiotic beverage.

Nutrients are invisible chemicals in the food which are necessary for keeping the body healthy. On the nutritional side, fermentation helps in degradation of anti- nutritional factors and increases bioavailability, protein digestibility and degradation of flatulence causing oligosaccharides.

The nutritional assessment of the probiotic beverage was carried out with respect to energy, carbohydrates, proteins, vitamin C and minerals such as iron, sodium, potassium and calcium. Energy content of the probiotic beverage was estimated as 288kcal/100g which was slightly higher than the non probiotic beverage of 256kcal/100g. Neelofer (2004) also reported that energy content of in malted health drink mix was 332kcal/100g and that of therapeutic health drink mix was 335kcal/100g. Carbohydrate content of probiotic beverage was estimated as 72.66 g /100 g of sample, which was slightly higher than non probiotic beverage with a carbohydrate content of 64.66 g /100 g. Protein content (0.082g/100g) and vitamin C (0.072 mg /100g) was negligible in the probiotic beverage. Suma (2009) reported that vitamin C content of dehydrated banana drink mix was negligible. Shukla (2013) reported that whey pineapple juice probiotic beverage with protein content of 0.23%

Mineral content of the probiotic beverage was also assessed Iron content of the developed beverage was assessed and it was clear that iron content of 0.59 mg/ 100 g of sample which was higher than the non probiotic with an iron content of 0.25 mg/100g.Sodium content of probiotic beverage was 1.5mg/100g while non probiotic beverage had a sodium content of 1.1. Likewise potassium content of probiotic beverage was 0.5mg/100g but the non probiotic beverage had potassium content of 0.4mg/100g. Health drink mix prepared from jack fruit seed flour recorded higher potassium content of 170- 387 mg/100g of (Shruti, 2005).

In the present study by the probiotication, the calcium content of the beverage increased to 3.2mg/100 from 2.6 mg /100g. Suma (2009) found out that calcium content of dehydrated banana drink mix ranged from 14.35- 33.16 mg/100g.

Results of present study clearly revealed that nutrient composition of the probiotic beverage enhanced due to probiotication. It may be due to the bioavailability of nutrients and can be said that by the probiotication the bioavailability of the nutrients in the beverage increased with probiotication.

It can be concluded that probiotic beverage was outstanding in all the aspects such as sensory, chemical and nutritional qualities in comparison with non probiotic beverage.

5.5 Shelf stability of probiotic beverage

The beverage was stored in glass bottles for storage study. Shelf stability of the probiotic beverage was assessed by storing beverage at ambient and refrigerated condition. Probiotic beverage stored at ambient condition was monitored every day while beverage stored at refrigerated condition was monitored alternate days. Sensory qualities, changes in the chemical constituents and viable count of the probiotic organisms were the parameters ascertained for shelf stability of the beverage. The viability of probiotic organisms was dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature (Shah, 2000).

5.5.1 Beverage stored at ambient condition

5.5.1.1 Changes in the sensory qualities of probiotic beverage

Sensory qualities decreased drastically when kept under ambient condition. Percentage decrease for appearance was 58.33, while for colour, flavour, taste and consistency were 24, 64, 74.47, 56.36. Overall acceptability of the beverage has also altered drastically to 63per cent(Fig4). By the end of third day the beverage was not able to relish by the panelist. It may be noted that decrease was more pronounced with respect to flavour and taste followed by appearance and consistency.

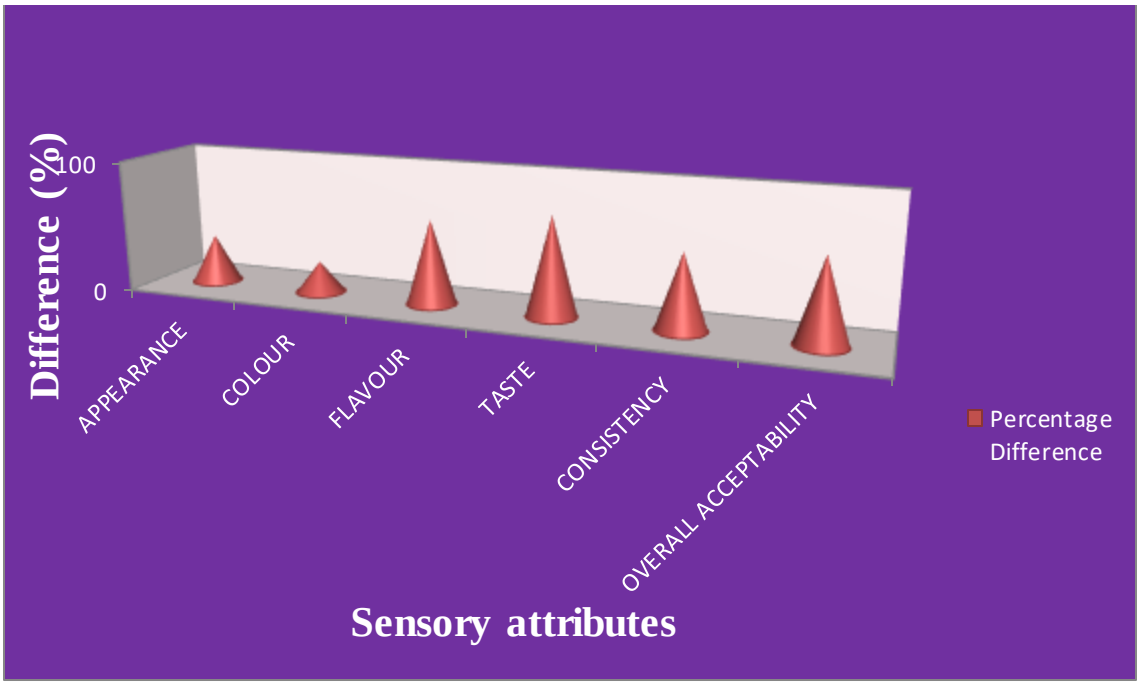


Fig 4: Changes in sensory attributes of probiotic beverage when stored under ambient condition

According to a consensus made with the judges during sensory evaluation, it was determined that the main descriptors that characterized the product were acidity and sweetness, with acidity being the character responsible for the sensory difference perceived by the members. Shukla *et al.* (2013) observed the percentage decrease in the sensory attributes such as colour and appearance, flavour, consistency and overall acceptability of the whey based pineapple juice probiotic beverage were 50.35, 65.25, 53.29 and 54.64. Junaid *et a*l. (2013) reported that flavoured acidophilus milk was rated about 7.8 in hedonic rating by the panelists, which was decreased to about 6.6 after 6 days of storage. These findings supported that sensory scores of the probiotic drink decreased when stored under ambient condition which was seen in the present study also.

These findings confirmed that, probiotic beverage is not advisable to store at ambient condition. This may be due to the fact that once desired viable count is obtained and if it was not arrested; further multiplication takes place, which produce undesirable sensory profile in the product.

5.5.1.2 Changes in the chemical constituents of the probiotic beverage

Chemical constituents of the beverage stored under ambient condition revealed that there was a tremendous reduction in the chemical constituents also.

The TSS of the probiotic beverage stored at ambient condition slightly increased from 23^0- 26.0^0 brix from first to third day(Fig5a). It may be due to the increase in the rate of fermentation. This increase was found significant at 5 percent level.

Acidity of the probiotic beverage increased considerably from 0.07% - 0.32%.(Fig5c) Significant difference at 5 percent in the acidity was noted in the beverage at 5%. Shukla *et al.* (2013) reported that increase in acidity of the beverage was more prominent when stored at ambient temperature where acidity reached 0.89% after 120 hr of storage While pH of the developed probiotic beverage decreased from 6.5 – 6.3 from first to third day(Fig5b). Yoon *et al.* (2005) reported

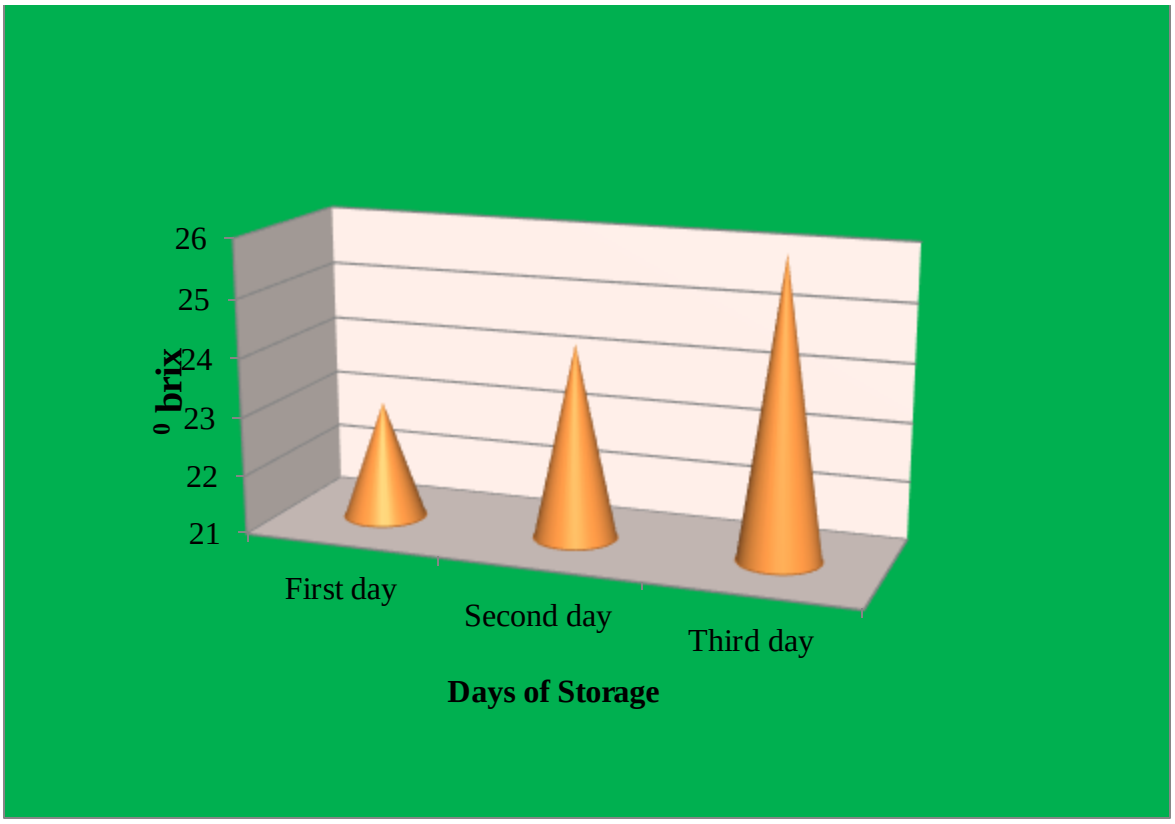


Fig 5(a): Changes in TSS of probiotic beverage stored under ambient condition

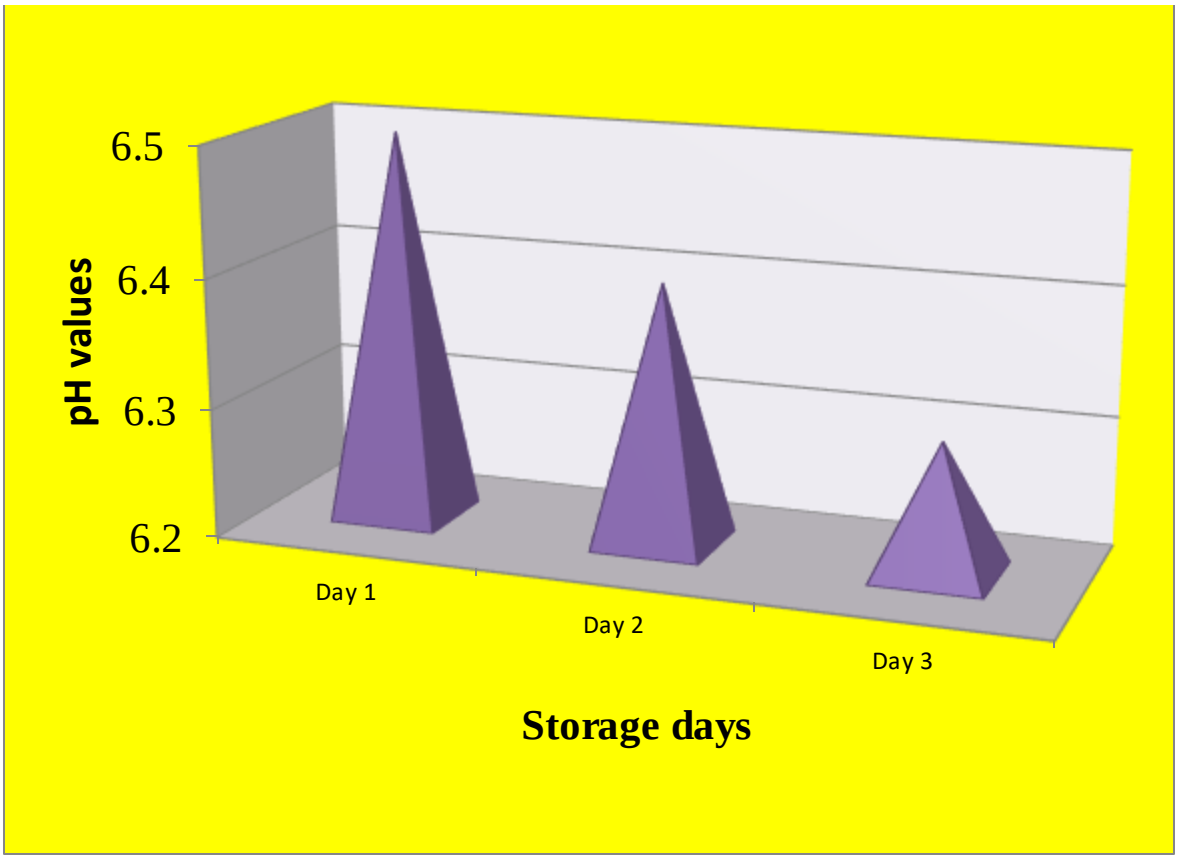


Fig 5(b) : Changes in pH of probiotic beverage stored under ambient condition

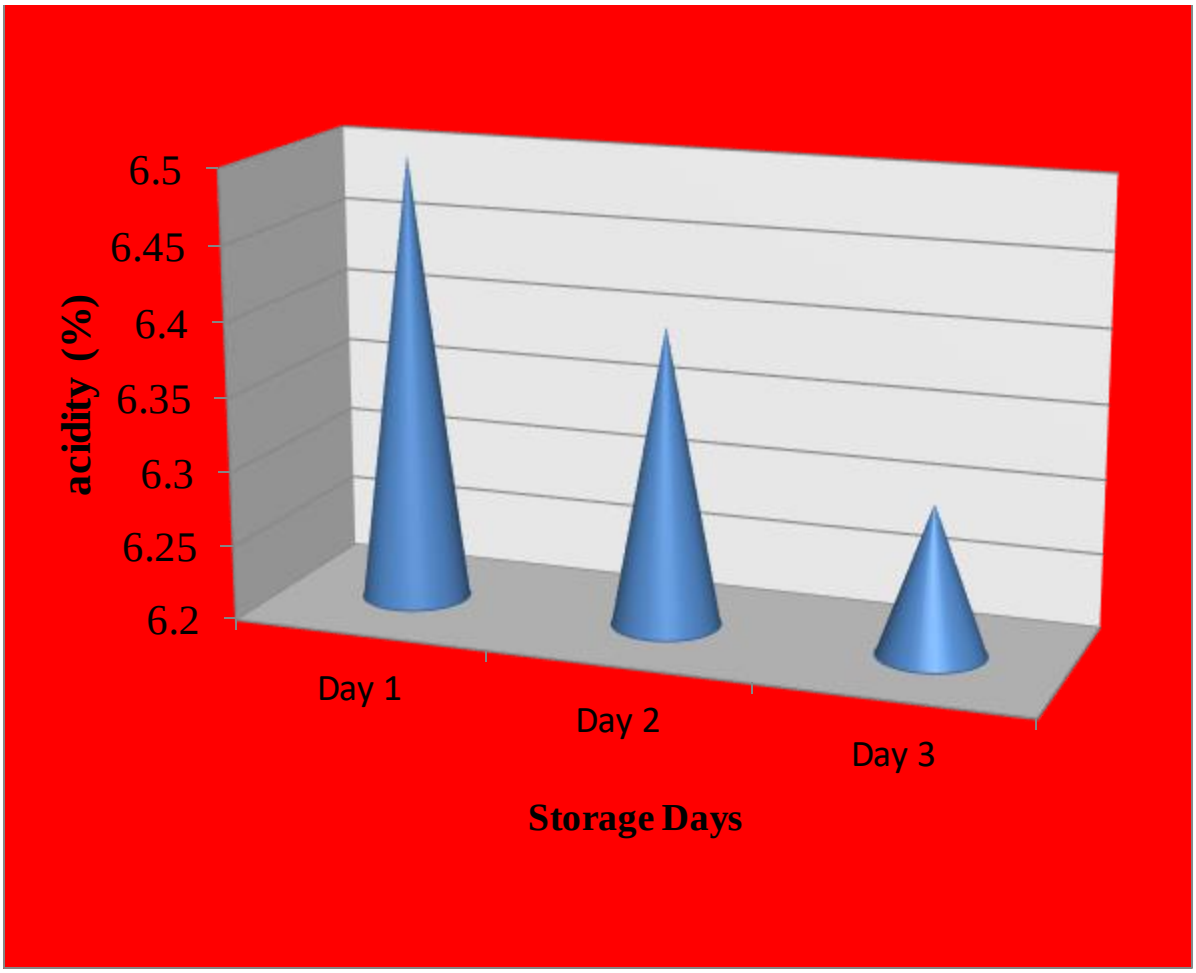


Fig 5(c) : Changes in acidity of probiotic beverage stored under ambient condition

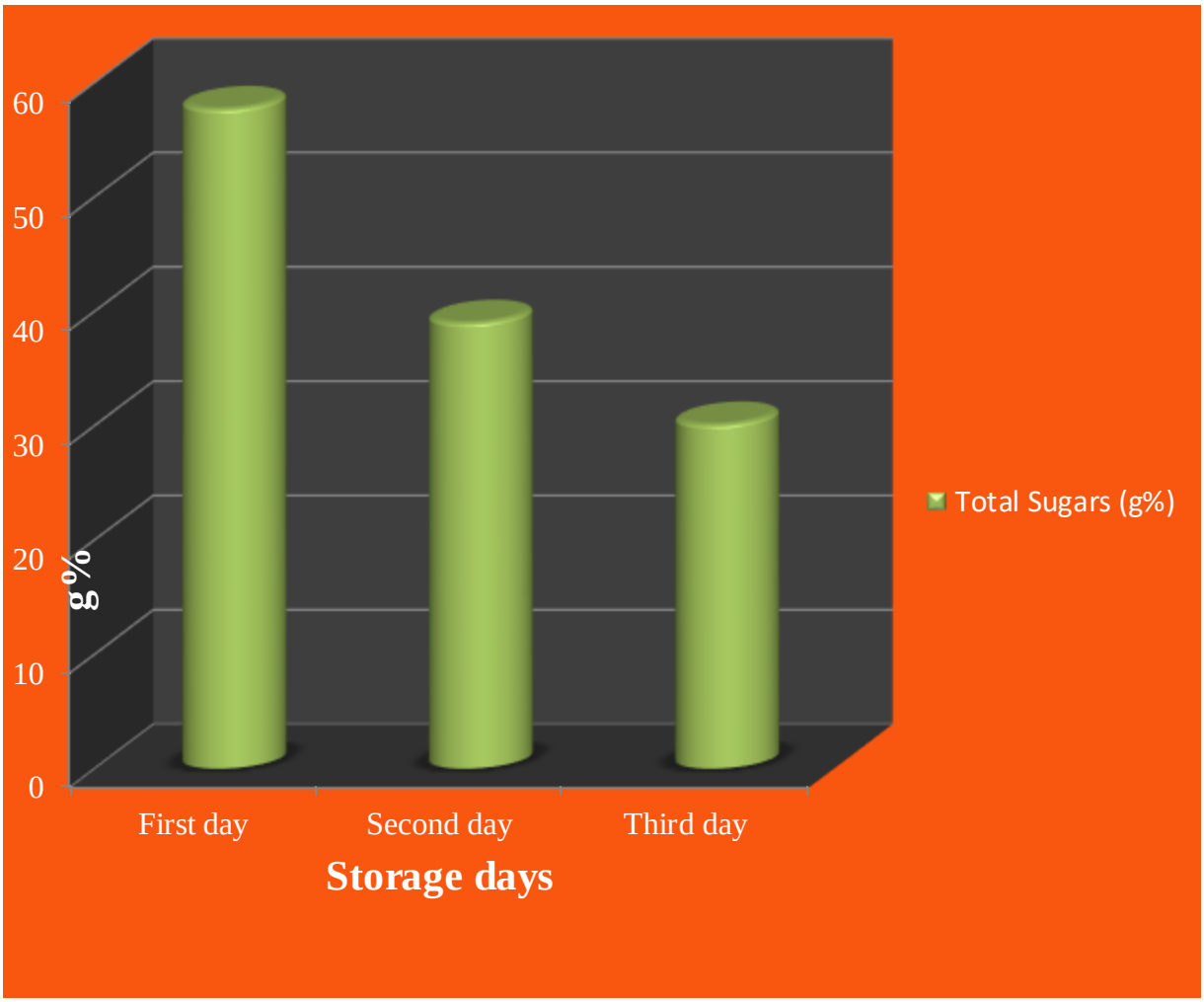


Fig 5(d) : Changes in total sugars of probiotic beverage stored under ambient condition

that *L.acidophilus* and *L. plantarum* reduced the pH of beet juice from 6.3-4.5 after 48 hours of fermentation due to their ability to produce a greater amount of lactic acid Shukla *et al.* (2013) reported that during storage of probiotic beverage at ambient temperature, pH lowered significantly from 4.28- 3.90 after 48 hours.

The total sugar content of the probiotic beverage also decreased during storage at ambient condition from 57.7 – 30.12 g of glucose/100 g (Fig 5 d). Tiwari and Deen (2014) reported that total sugars of bael and aloevera RTS increased continuously from 10.21% - 11.12% during entire storage period. Increase in sugar content with the advancement of storage period was observed in karonda squash (Deen and Singh., 2012) stored at room temperature.

5.5.1.3 Changes in viable count in the probiotic beverage

The viability of probiotic organisms is dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature (Shah, 2000).

The viable count of the probiotic beverage was assessed during storage at the ambient condition and showed a tremendous decrease in the viable count day by day. The initial viable count of 74.4 x 10^9 cfu/ml, was decreased to 59.4 x 10^9 cfu/ml after 24 hours and decreased further to 33x 10^9 cfu/ml by 48 hours and then it decreased to 21.4x 10^9 cfu/ml after 72 hours (Fig6).

Acidity, the acid production during storage (post-acidification), the oxygen level, the package permeability to oxygen, the susceptibility to antimicrobial substances produced by bacteria and the lack of some nutrients in milk are very important factors that may lead to significant loss of probiotic activity during storage (Kailasapathy and Chin, 2000).

Junaid *et al.* (2013) reported that viable count of *Lactobacillus acidophilus* in different flavoured milk found to vary from first day to sixth day. At first day viable count of mango, pineapple and strawberry flavoured milk were 2.50x 10^6cfu/ml, 3.60x 10^6 cfu/ml and 2.87x10^6 cfu/ml to 1.50x 10^6 cfu/ml, 3x 10^6 cfu/ml

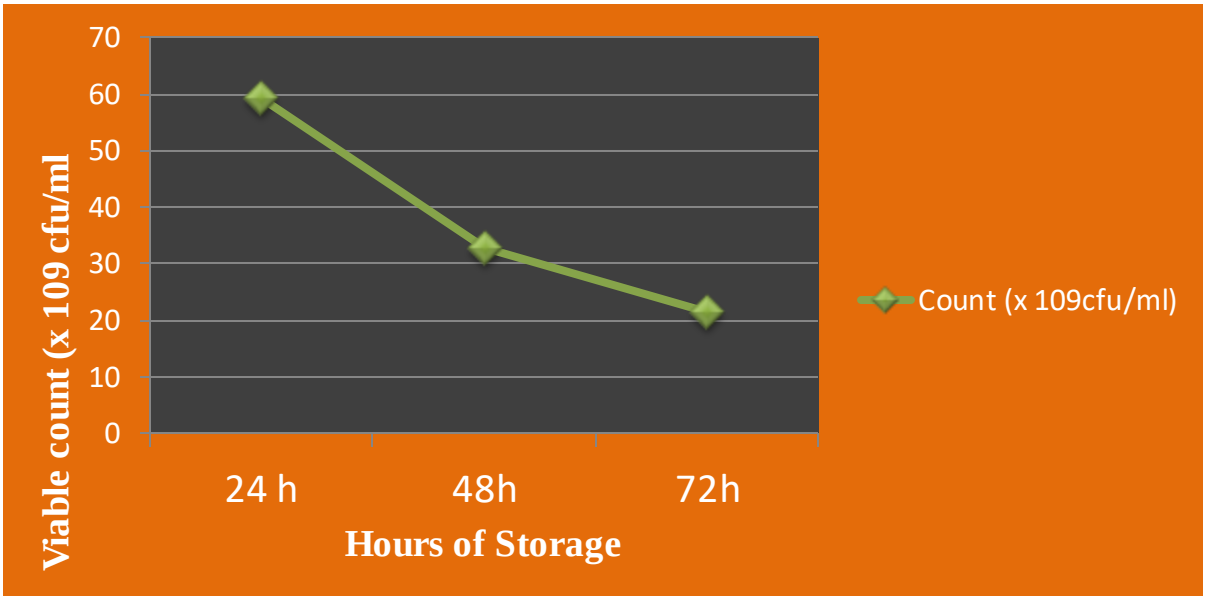


Fig 6: Changes in viable count in probiotic beverage during storage under ambient condition

and 2.50x 10^6 cfu/ml after 6 days. However, the total viable count was under acceptable range even after 6 days of storage indicating good keeping quality up to 6 days.

Shukla *et al.* (2013) reported that during storage of whey pineapple juice probiotic beverage at ambient condition the total viable count first increased to 9.5×10^8 (in 48 hr) and then gradually declined to 2.9×10^7 cfu/ml after 120 hr (5 days).

In this context, Epsinoza (2010) reported that decrease in the pH of the medium and accumulation of lactic acid, diacetyl, and acetaldehyde from growth and fermentation are the main factors for viability loss of probiotics added.

Results clearly depicted that the beverage was not fit to consume if stored at ambient condition. It can be inferred that the ambient condition is not proper for the shelf life of the probiotic beverage as it affects the sensory qualities, chemical constituents and viable count in 72 hours.

5.5.2 Beverage stored under refrigerated condition

5.5.2.1 Changes in the sensory quality of the Probiotic beverage

Based on the sensory analysis of the probiotic beverage stored under refrigerated condition clearly indicated that till the eighth day of storage all the sensory attributes remained constant. However mean ranks secured for different sensory attributes of beverage viz, appearance, colour, flavour, taste, consistency and overall acceptability found to decline after eight days and their percentage decrease were 76.90, 76.53, 85.74, 87.40, 74.10 and 73.90 by fifteenth day (Fig7). Sensory attribute of the probiotic beverage reduced by fifteenth day. Still the panelists expressed that beverage was acceptable to consume till tenth day.

Shukla *et al.* (2013) reported that whey based probiotic beverage did not show negligible sensory differences for the first two weeks at refrigerated storage and judges agreed that the beverage was acceptable for a period of 24 days at refrigerated condition.

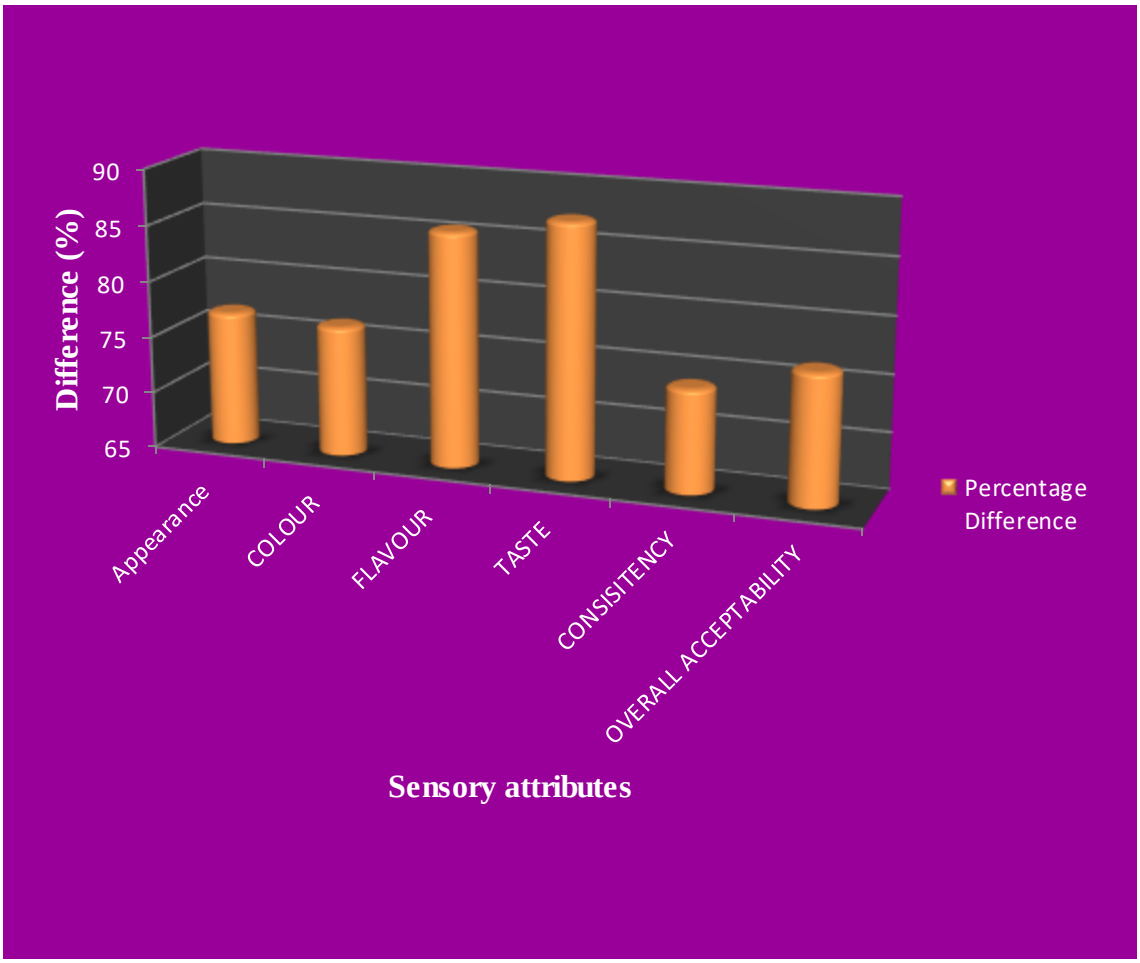


Fig 7: Changes in sensory attributes of probiotic beverage stored under refrigerated condition

Daneshi *et al*. (2013) found that milk/ carrot Juice drink inoculated with *L. acidophilus, B. lactis* and *L. plantarum* showed higher sensory acceptability over 20 days storage. Charanjiv *et al*. (2006) who showed that probiotic carrot flavoured milk remained in good condition for 4 days under refrigeration.

5.5.2.2 Changes in the chemical constituents of the probiotic beverage

The chemical analysis of probiotic beverage in refrigerated condition revealed that TSS of the probiotic beverage during storage increased from 24^0 brix to 31^0 brix (Fig8a).pH of the beverage remained stable till 8 days after which it showed declining trend . Similar results were recorded by researchers also(Fig8b). Shukla (2013) reported that probiotic beverage from whey and pineapple juice gradually declined after 12 days of refrigerated storage and the pH ranged from 4.38 to 3.98 after 28 days of storage. Kumar and Manimeghalai (2003) reported a decline in pH of whey based pineapple probiotic beverage after 19 days at refrigerated storage. Daneshi *et al*. (2012) reported that during cold storage of carrot fortified milk, pH did not show significant change in first week but it was decreased to 5.85 in 3rd week. This might be due to the degradation of lactose or produced galacturonic acid and other acids by enzymatic breakdown of pectin (Charanjiv *et al*.,2006). Kumar *et al*. (2004) showed that there was a gradual decline in pH of soy milk and whey blended papaya RTS.

Acidity of the probiotic beverage ranged from 0.07 to 0.23 during storage of 15 days at refrigerated condition(Fig8c) .With respect to acidity, a significant increase was recorded and was significant at 5% level. Similar results were recorded by other researcher also. Shukla *et al.* (2013) reported that acidity of the probiotic beverage from whey and pineapple juice increased during the refrigerated storage from 0.546 to 0.89% after 28 days.

The total sugar content of beverage at storage in refrigerated condition decreased from 40.0 - 25 g of glucose/100g (Fig8d). However,

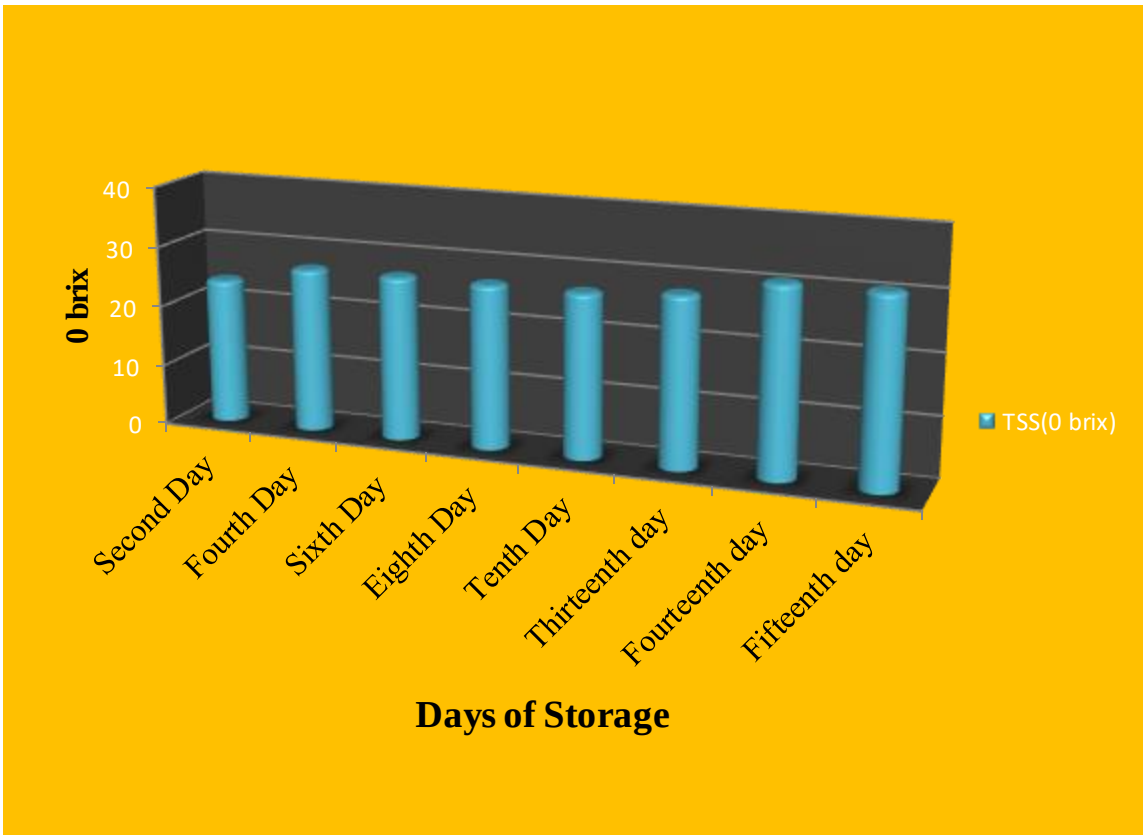


Fig 8 : Changes in TSS of probiotic beverage stored under refrigerated condition

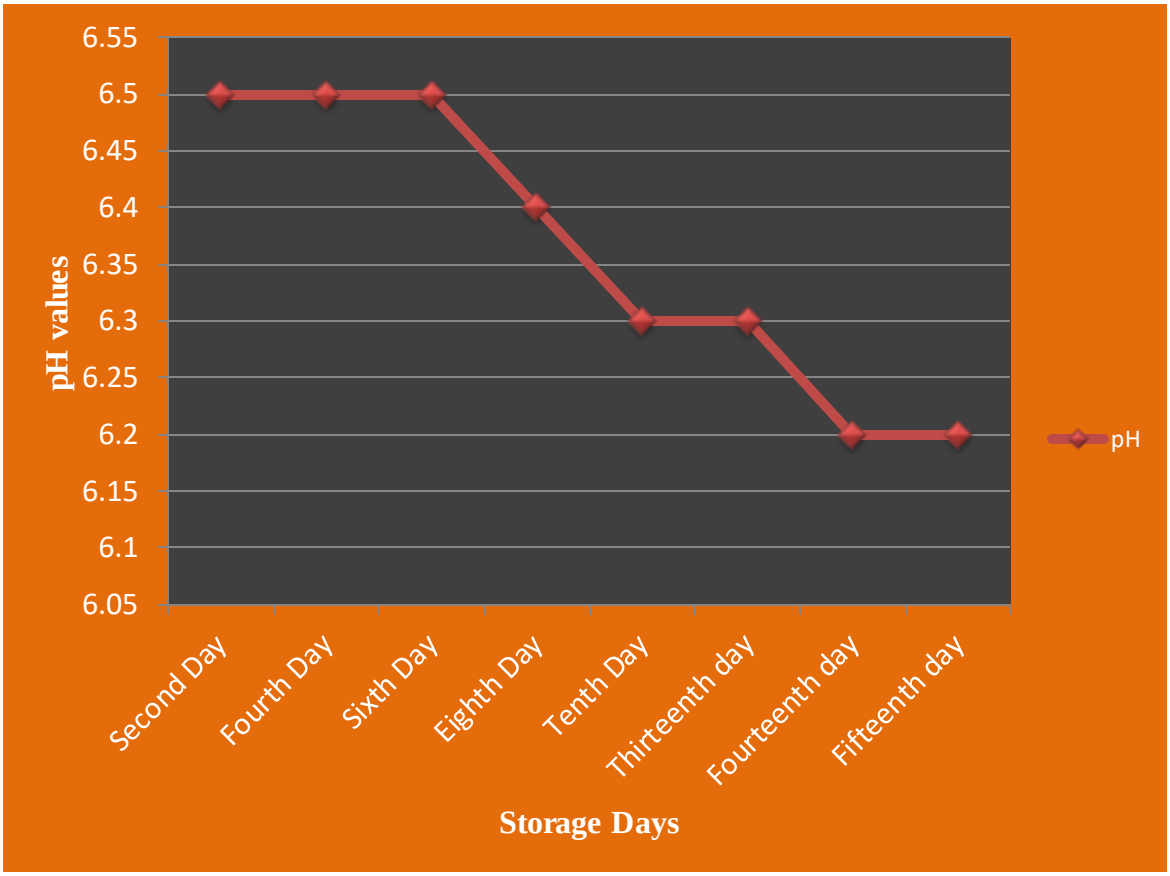


Fig 8(b) : Changes in pH of probiotic beverage stored under refrigerated condition

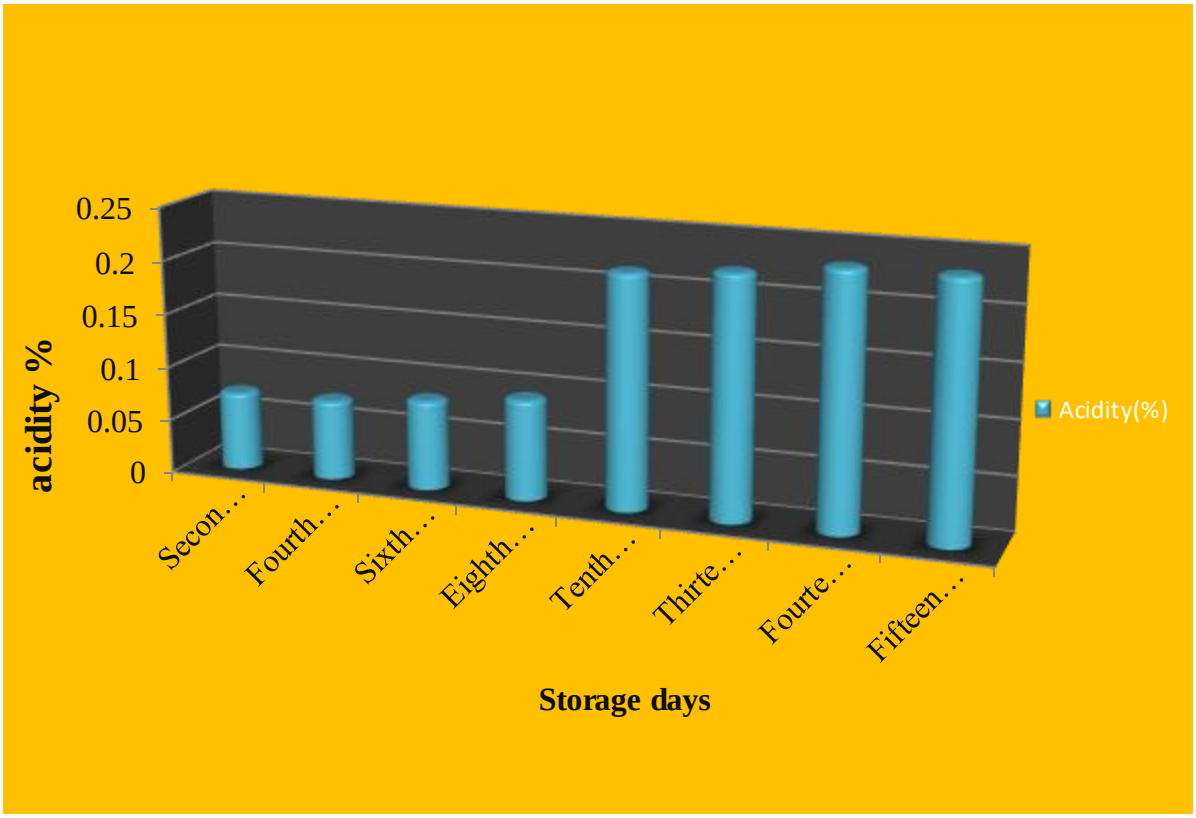


Fig 8(c) : Changes in acidity of probiotic beverage stored under refrigerated condition

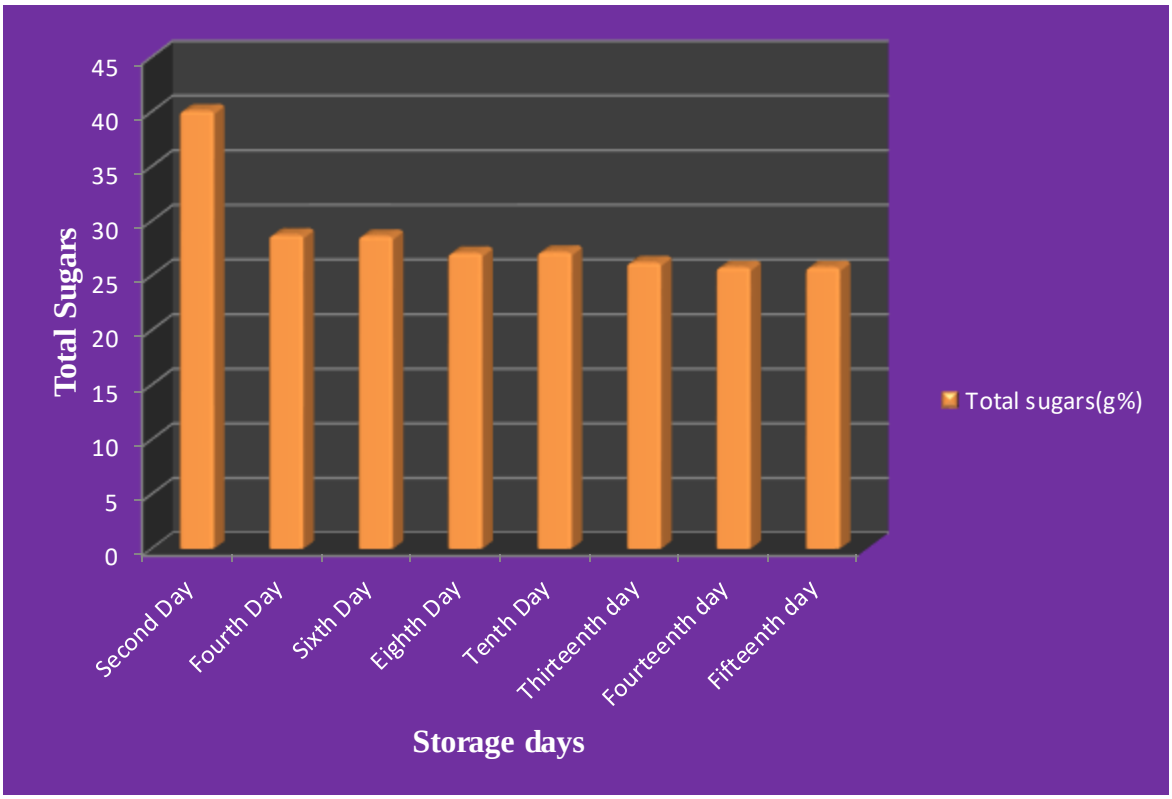


Fig 8(c): Changes in total sugars of probiotic beverage stored under refrigerated condition

Bhagwan and Awadhesh (2014) observed that TSS, acidity, reducing sugars and total sugars content increased on storage . The findings are in consonance with the earlier studies reported in bael and guava blended RTS (Nidhi *et al*., 2008) and in karonda squash (Deen and Singh, 2012).

5.5.1.3 Changes in the Viable count in the probiotic beverage

Viable count of *Lactobacillus acidophilus* in beverage stored in refrigerated condition increased till fourth day of storage from $21.2x10^9$ cfu/ml to $42.4 x10^9$ cfu/ml, while on 6^{th} day it declined to $33.6 x 10^9$ cfu/ml and thereafter it increased and on the fifteenth day it reached maximum viable count of $94.8x 10^9$ cfu/ml(Fig9).

Mattilla –Sandholm (2002) opined that decline in viable count in between storage days due to low pH and high acidity in fermented beverages. Oxygen tension and water activity are two important characteristics of fruit juices that lead to weak growth ability of probiotic bacteria (Yoon *et al*., 2004).

Shukla *et al.* (2013) observed that the initial total viable count of the beverage was 3.8×107 cfu/ ml which decreased to $1.8×10^7$ at refrigerated storage. Although the viability of *Lactobacillus acidophilus* population decreased, the viable count of the probiotic beverage did not fall below 10^6 cfu/ml during 24 days of refrigerated storage.

Yoon *et al*. (2004) reported that *L. acidophilus* in the fermented beet juice remained at 10^6- 10^9 cfu/ml after 4 weeks of cold storage at 4^0 C. It is important to have a significant number of viable lactic acid bacteria present in the finished product for maximum health benefits (Shah, 2001). Yoon *et al* .(2006) developed probiotic cabbage juice using by inoculating 24 hour old culture at 30^0C of *L. plantarum, L. casei and L. delbrueckii* and viable count reached 1 x 10^9 cfu/ml after 48 hours of fermentation. Angelov *et al*. (2006) formulated probiotic tomato juice with shelf life of 21 days at refrigerated condition. Pakbin *et al*. (2014) standardized

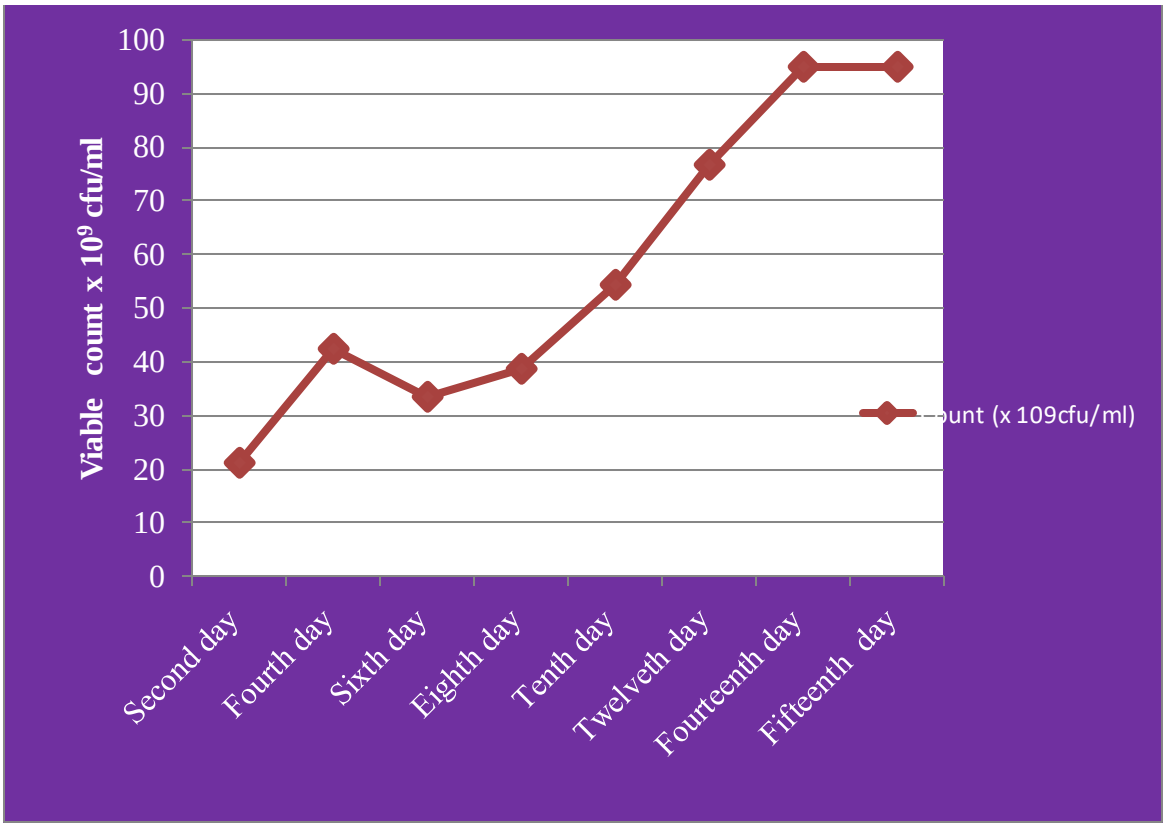


Fig 9: Changes in viable count of *Lactobacillus acidophilus* in the beverage during storage at refrigerated condition

probiotic peach juice with shelf life of one week (7 days) under refrigerated condition.

Charanjiv *et al*. (2006) showed that carrot flavoured milk remain in good condition for 4 days under refrigerated condition. Yoon *et al*. (2006) observed that probiotic cabbage juice has a shelf stability of 2 weeks of cold storage and could serve as a healthy beverage for vegetarians and lactose- allergic consumers.

Mortazavian *et a*l. (2007) reported that highest viability of *Lactobacillus acidophilus* was observed in yogurt after 20 days of cold storage (2^0C). Pereira *et al*. (2010) reported that viable counts of *L. casei* in probiotic tomato juice were higher than 8.00 Log cfu/ml during storage of 42 days.

Whey pineapple based probiotic beverage was reported to have shelf life of 21 days (Shukla *et al*., 2013). Antimicrobial substances produced by probiotics such as bacteriocins or organic acids and competition with other microorganisms in product may be known as reasons of longer shelf life of probiotic samples than control (Oelschlaeger, 2010). .

From the results, it can be concluded that the beverage though slightly declined in the sensory, chemical parameters, viable count maintained at a desirable level. So the developed beverage could be consumed within 10 days from its manufacture if stored under refrigerated condition. This could be further enhanced by adopting modern technologies. Cruce and Goulet (2001) reported that microencapsulation technology could be utilized to coat probiotic bacteria can extend shelf life, of the probiotic beverage.

5.6 Consumer acceptance of the probiotic beverage

Functional Foods (FFs) represent one of the most interesting areas of research and innovation in the food industry (Siro *et al*., 2008). In this context probiotic food products contribute much.

In the present investigation, consumer acceptance of the probiotic beverage was assessed by hedonic rating by 30 consumers selected from children,

adolescents and professionals using nine point scale. Ninety percent of the consumers recorded probiotic beverage with a rating 'like extremely to like very much' as against 69 percent to the non probiotic beverage. This itself is an indication of well acceptance of the developed probiotic beverage among the consumers. All the sensory attributes enhanced by the process of probiotication and the study clearly indicated that health promoting food products are readily acceptable to the consumers.

5.7 Cost and Yield ratio of the probiotic beverage

Cost of the product is very important, as the product cost can decide its profit or loss. Cost of the product depends on the cost of the raw materials, and other inherent cost involved in the formulation of the beverage.

Cost of the beverage was computed and found as Rs 15/- per 100 ml and when compared with the commercially available probiotic products cost was comparatively less such products at present available in market was found to be

Rs 25- 50/100 ml.

Yield ratio of the probiotic beverage was found as 0.83 and only a negligible loss was accounted in the formulation of the beverage. Thus it is inferred that the beverage is cost effective also.

SUMMARY

SUMMARY

The present study entitled "Development and quality evaluation of probiotic honey beverage" was carried out during the year 2014-2015. The main objective of the study was to develop a probiotic honey beverage and to evaluate its chemical, nutritional, organoleptic and shelf life quality.

The major ingredients used for the formulation of the honey beverage were honey (*Apis cerana indica*), aloevera pulp and soymilk. Honey was procured from local honey growers, aloevera leaves plucked from clean and hygienic well maintained fields, while soymilk was procured from the local markets. Aloevera pulp was extracted by grinding aloevera gel.

The different combinations of beverage were formulated by blending various ingredients in different proportions by "trial and error method". Out of the twelve combinations, nine combinations were subjected to sensory evaluation using score card and by hedonic rating.

Based on the rank means obtained for sensory quality three combinations, which has got maximum mean ranks were selected for in depth analysis. The combination prepared with equal quantity of honey and soymilk (25 percent each) with 10% of aloevera has obtained maximum mean ranks. The proportions of honey, aloevera pulp, soymilk and water used for the formulations of the other two combinations were 3:1:2:4; 4:1:1.5:3.5.

The mean ranks obtained for the best combination (C_9) for various sensory attributes such as appearance, colour, flavour, taste, consistency and overall acceptability were 67.6, 69.0, 69.2, 72.9, 75.0 respectively. Thus C_9 combination adjudged to be the best, followed by C_8 with mean ranks 59.2, 58.8, 62.0, 70.1, 68.5: and C_6 with mean ranks 55.0, 55.2, 58.4, 61.5, 58.4. The mean ranks for overall acceptability of three combinations C_9, C_8 and C_6 were 69.5, 66.0 and 59.0.

The selected combinations were subjected to flavour enhancement process with four flavours viz lime, chocolate, vanilla and cardamom. The overall acceptability scores obtained for the best combination (C_9) for flavoured beverage were 9.8, 19.1, 20.1 and 20.7 respectively for lime, chocolate, vanilla and cardamom. The results confirmed that cardamom is the best flavour for all the three combinations.

The chemical analysis of the three combinations was carried out with respect to their TSS, pH, acidity, reducing sugars and total sugars. TSS of the Combinations ranged from 20^0 brix to 23^0 brix. While pH of the formulations ranged from 4.4- 6.5, acidity of the beverage ranged from 0.062-0.082%. Reducing and total sugars of the formulations were in the range of 26.55g% -32.0g% and 93.98g%-73.09g% respectively.From the analysis of the chemical constituents it was found that pH and TSS was higher for the C_9 combinations, while acidity, reducing and total sugars were higher for the C_6 combination.

The nutrient constituents of the formulations were also ascertained with respect to their energy, carbohydrate, protein and vitamin C and minor nutrients such as sodium, potassium, iron, calcium and total minerals. The energy content of the formulations ranged from 256-326kcal/100g, while carbohydrate ranged from 64.66-81.33g/100g. Protein and vitamin C were present only in very negligible amount. The minor nutrients such as iron, sodium, potassium, calcium and total minerals are in the range of 0.25-0.87mg/100g;1.1-2.3 mg/100g;0.4-0.8mg/100g and 230-280mg/100g and 1.5-2.5% .

From analysis it can be concluded that chemical as well as the nutrient constituents vary with the three combinations and Combination with low pH (C_6) was discarded.

Lactobacillus acidophilus was the culture used for probiotication process. The procured culture was sub cultered in MRS medium with different pH. After the selection of the culture, optimization of pH for the growth of organism

was finalized. Culture was inoculated at varying pH from 4.0- 7.0 and found that growth was adequate with a pH from 6.2- 6.6. In the present study, since the culture depicted good growth at a pH 6.5, the combination C_9 was finally selected and the pH was optimized at 6.5. C_8 combination with pH 5.5 was discarded, and further process was proceeded with C_9 combination.

Three doses viz, 1.0 1.5 and 2.0 per cent inoculum was inoculated and viable count was recorded at varying time interval of 3 hours, 6 hours and 9 hours. The maximum viable count obtained by 1.0 per cent inoculum was 85.8 x 10^9 cfu/ml, 1.5 per cent inoculum attained viable count of 115 x 10^9 cfu/ml and 2 per cent inoculum depicted maximum viable count of 135x 10^9 cfu/ml at 6 hours of incubation. The viable count of *lactobacillus acidophilus* (1 per cent) in beverage at 3hours, 6 hours and 9 hours of incubation were 46x 10^9 cfu/ml, 85.8 x 10^9 cfu/ml and 64.4 x 10^9 cfu/ml. In the present study the dosage was optimized to 1% since the viable count obtained at 6 hours of incubation itself the recommended level of 10^6- 10^9 cfu/ml. .

Standardised beverage was subjected to sterilization at 100^0 C and 80^0C. Sterilization at 100^0 C was not adviced due to alteration in the sensory parameters mainly colour of the beverage and become intense besides curdling occurred in the beverage When the beverage was sterilized at 80^0 C, at this temperature also Colour change and appeal was decreased. Thus it was decided to sterilize individual ingredients separately before blending except honey.

Finally the beverage was formulated using honey, soymilk and blended with sterilized Aloevera pulp and water before the probiotication. Enumeration of viable count of *Lactobacillus acidophilus* at 6 hours of incubation indicated 95.8x 10^9 cfu/ml which was more than enough for desired health benefits.

The quality of the probiotic beverage was ascertained with respect to their sensory attributes, chemical constituents and nutritional constituents.

The scores obtained for the parameter appearance of Probiotic and non probiotic are 4.6 and 4.1 respectively. The average score obtained for colour of probiotic beverage was 4.7 as against 4.1 in the control beverage. Probiotic beverage has recorded an average score of 4.6 and that of non probiotic was 4.1 for flavour. Probiotic beverage has recorded more acceptances in the taste attribute (4.5) compared to non probiotic beverage (4.0). It was found the probiotic beverage was thicker and cloudier than the control and scored 4.7 for consistency as against 4.1 in the non probiotic beverage. Probiotic beverage has got higher overall acceptance of 4.5 out of 5.

By the probiotication process, TSS increased from 23^0 brix to 24^0 brix, pH increased to 6.6 from 6.5, acidity increased to 0.083% from 0.076%, reducing sugars and total sugars increased to 35g/100g from 29.40g% and 86.20g% from 78.11g% respectively. The nutrient constituents such as energy increases to 288kca/100g from , carbohydrate (72.66g/100g),while protein content (0.80%) and vitamin C (0.072mg%) of the probiotic beverage were higher than the control beverage. The mineral such as iron (0.59 mg/100g) and calcium (320 mg/100g) were significantly higher than the control beverage and no significant change in sodium and potassium content.

The shelf life of the probiotic beverage was ascertained by storing the beverage under ambient condition. It was found that product cannot be stored at ambient as its sensory parameters and chemical constituents changes by the third day. Percentage decrease for sensory attributes of the beverage stored under ambient condition such as appearance was 58.33, while for colour, flavour, taste and consistency were 24, 64, 74.47, 56.36. Overall acceptability of the beverage has also altered drastically to 63%.

The TSS of the probiotic beverage stored at ambient condition slightly increased from 23^0- 26.0^0 Brix from first to third day, while increased considerably

from 0.07% - 0.32%. The total sugar content of the probiotic beverage also decreased during storage at ambient condition from 57.7 – 30.12 g of glucose/100 g.

The viable count was 74.4 x 10^9 cfu/ml, at incubation day, was decreased to 59.4 x 10^9 cfu/ml after 24 hours and decreased further to 33x 10^9 cfu/ml by 48 hours and then it decreases to 21.4x 10^9 cfu/ml after 72 hours.

It can be inferred that the ambient condition is not proper for the shelf life of the probiotic beverage as it affects the sensory qualities, chemical constituents and viable count in 72 hours.

Mean ranks secured for different sensory attributes of beverage viz, appearance, colour, flavour, taste, consistency and overall acceptability found to decline after eight days and their percentage decrease were 76.90, 76.53, 85.74, 87.40, 74.10 and 73.90 by fifteenth day. Sensory attribute of the probiotic beverage reduced by fifteenth day.

TSS of the probiotic beverage during storage increased from 24^0 brix to 31^0 Brix. pH of the beverage remained stable till 8 days after which it showed declining trend, acidity of the probiotic beverage ranged from 0.07 to 0.23 during storage of 15 days at refrigerated condition, while total sugar content of beverage at storage in refrigerated condition decreased from 40.0 - 25 g of glucose/100g.

Viable count of *Lactobacillus acidophilus* in beverage stored in refrigerated condition increased till fourth day of storage from 21.2x10^9 cfu/ml to 42.4 x10^9 cfu/ml, while on 6^{th} day it declined to 33.6 x 10^9 cfu/ml and thereafter it increased and on the fifteenth day it reaches maximum viable count of 94.8x 10^9 cfu/ml. The beverage has a shelf life of ten days under refrigerated condition with moderate viable count and good sensory attributes.

The consumer acceptance of the probiotic beverage was carried out among 30 people using hedonic rating on nine point scale and it was found that 91 percent consumers "like extremely to like very much" probiotic beverage as against

69 percent to the non probiotic beverage. Thus a consumer acceptable probiotic beverage was formulated with shelf life of ten days under refrigerated condition.

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ABSTRACT

DEVELOPMENT AND QUALITY EVALUATION OF PROBIOTIC HONEY BEVERAGE

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Abstract of the thesis

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ABSTRACT

The study, entitled "Development and quality evaluation of probiotic honey beverage" was carried out with the main objective to develop a honey based probiotic beverage and to evaluate its chemical, nutritional, organoleptic and shelf life quality.

Honey, aloevera and soya milk were the ingredients utilized for the formulation of the product. Various combinations (C_1- C_9) with different proportions of ingredients were blended by "trial and error method" to obtain an ideal, consumer acceptable beverage. The percentage of various ingredients viz honey, aloevera pulp, soya milk and water in the C_1- C_9 combinations were 50-25 per cent, 20-10 per cent, 15-25 per cent and 15-40 per cent respectively. Based on the sensory quality (rank means and hedonic rating) three proportions viz C_6, C_8 and C_9 were selected for probiotication process. Overall mean rank scores for the selected combination were 69.5, 66 and 59 respectively for C_9 C_8 and C_6.

Selected combinations were subjected to chemical and nutritional quality analysis. TSS, pH, acidity, reducing sugars and total sugars of the selected combinations ranged between 20^0 Brix - 23^0 Brix, 6.5-4.4, 0.08% to 0.06%, 29-32 g /100g and 73-93 g/100g respectively. While energy and carbohydrate content of the three combinations ranged from 258 – 325 kcal/100g and 64 – 81 g/100g. Negligible protein and vitamin C was recorded in the selected combinations. With respect to mineral content, among selected combination C_6 was having maximum content of iron(0.059mg/100g), sodium (2.3mg/100g), and potassium (0.8mg/100g) while C_9 recorded higher calcium content of 3.2mg/100g.Combination with lowest pH was discarded and C_8 and C_9 maintained for probiotication process

Lactobacillus acidophilus was the culture used for the probiotication process. pH, dosage of inoculum and incubation period were optimised based on the prescribed viable count in the probiotic beverage. Pre treatments prior to

probiotication process were also attempted in the study. Results confirmed that 1% inoculum at pH 6.5 with 6 hours of incubation period brought desirable level of viable count in the beverage (85.8 x 10^9 cfu/ml). Sterilization of individual ingredients in the beverage enabled to obtain a probiotic beverage free of pathogenic organisms with good sensory appeal.

The probiotic beverage developed was subjected to sensory, chemical and nutritional analysis in comparison with non probiotic beverage. Overall acceptability of the beverage was 4.5/ 5 with hedonic rating of 8.1.The chemical constituents such as TSS, acidity, pH, reducing sugars and total sugar of the probiotic beverage were recorded as 24^0 Brix, 0.083%, 6.6, 35.40 g/100g and 86.20g/100g respectively in the developed beverage and it was significantly higher than the control. Macro nutrients such as energy, carbohydrate and protein were 288 kcal/100g, 72.66g/100g and 0.082 g/ 100 g respectively.

The probiotic beverage depicted only three days of shelf life at ambient condition and 10 days at refrigerated condition. TSS and acidity enhanced during storage while total sugars decreased. The beverage was also found consumer acceptable (91%). Cost of 100 ml of probiotic beverage is Rs 15/- as against Rs 25-50/100 ml for similar marketed products. The cost of the developed probiotic honey beverage is found to be lower than the proprietary probiotic beverages in the market.

Considering the value addition of honey, the developed honey based probiotic beverage stands outstanding in sensory, chemical and nutritional quality and could promote health.

APPENDICES

APPENDIX I

DU0- Trio- Test

In the Duo Trio test, reference sample ‘R’ was given to the members. The members were asked to taste the sample carefully. Then a pair of coded sample was given to the members and asked to match with the reference sample ‘R’.

Name:

Product: Date:

Set No.	Code No. of Pairs	Same as ‘R’
1		
2		
3		
4		

Signature

APPENDIX II

SCORE CARD FOR HONEY BEVERAGE

Particulars	1	2	3	4	5	6	7	8	9
Appearance Very good -5 Good-4 Average-3 Poor-2 Very Poor-1									
Colour Very good-5 Good-4 Average-3 Poor-2 Very Poor-1									
Flavour Very Good-5 Good-4 Average-3 Poor-2 Very Poor-1									
Taste Very Good-5 Good-4 Average-3 Poor -2 Very poor-1									
Consistency Very Good-5 Good-4 Average-3 Poor-4 Very Poor-1									
Overall Acceptability Very Good-5 Good-4 Average-3 Poor-2 Very Poor-1									

APPENDIX III

HEDONIC RATING OF HONEY BEVERAGES

Particulars	**Scores**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Like Extremely	9									
Like Very Much	8									
Like Moderately	7									
Like Slightly	6									
Neither Like nor Dislike	5									
Dislike Slightly	4									
Dislike moderately	3									
Dislike Very Much	2									
Dislike Extremely	1									

Date: Name:

Signature: