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DEVELOPMENT OF PROBIOTIC JUICE

A PROJECT REPORT

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BONAFIDE CERTIFICATE

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ABSTRACT

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. The body, including the colon, is naturally filled with bacteria, yeast, and other microbes that work in symbiosis with the human body. These helpful bacteria enable healthier digestion and help fight infectious disease. Probiotics are similar in their effect and behaviour to these helpful bacteria, and play an important role in helping the body to gain or maintain a healthy internal ecosystem. The amount of "good bacteria" in the gut can be compromised by antibiotics or pathogenic microbes. When "unfriendly" yeasts, bacteria, or viruses colonize the colon, they can cause or contribute to many conditions, including ulcers, weakened immune system, irritable bowel syndrome, vaginal infection, and diarrhoea. Probiotics keep these problems at bay by stabilizing the balance of intestinal flora. Non-dairy probiotic foods are recently the main focus of research because of their various benefits. Fruit juices have been suggested as ideal media for probiotic growth because they inherently contain essential nutrients, good-looking and have good taste. Non dairy probiotic drinks are of recent trends due to vegetarianism, Cholesterol free and lactose intolerance. A probiotic fruit juice drink consisting of at least one species of probiotic bacteria chosen from Lactobacillus. The cucumber is a member of the Cucurbitaceous family. This vegetable is very high in water and very low in calories. It is an excellent source of potassium, vitamin C and folic acid. It is also a valuable source of potassium, sodium, magnesium, sulphur, silicon, chlorine and fluorine.

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LIST OF ABBREVIATIONS

C	Curd
GI	Gastro Intestinal Tract
IB	Idly batter
J	Juice
FAO	Food and Agriculture Organisation
MRS	De Mann Ragosa Sharpe media
RPM	Revolutions per minute
WHO	World Health Organisation
+	Poor growth of organism
++	Moderate growth of organism
+++	Good growth of organism
++++	Very good growth of organism

Introduction

CHAPTER 1

INTRODUCTION

Human intestinal flora contains as many as 10^{14} bacteria classified into 400-500 species, which are ten times higher than all the cells in the human body. Some bacteria of the intestinal flora such as *Clostridia, Proteus* and *Pseudomonas aeruginosa* can be harmful, while others like Bifidobacteria and Lactobacilli belonging to the so-called probiotic strains are favourable for the organism. The microflora in the large intestine plays an important part in the life of the host organism (Saxelin, 1996 and Lidbeck, 1993). Its composition may change several times during our life. However it can still be regarded as nearly constant.

Bacteria have been estimated to constitute 35-50% of the total of the human colon. The dominant genera are Bacteroides, Bifidobacterium, Eubacterium, Clostridium, Peptococcus, Peptostreptococcus and Ruminococcus. The subdominant genera include Escherichia, Enterobacter, Enterococcus, Klebsiella, Lactobacillus, Proteus, etc (Salminen and Tuomla, 1998). It has been found that every individual has hundreds of species of these genera with the combinations of the predominant genera species being uniquely different in each individual. Research suggest that there is a symbiotic relationship between the host and gut flora. The microbial inhabitants of the gut profoundly influence nutritional, physiologic, and protective processes. The bacteria exerts several benefits to the host such as breaking down of the food remains that have not been digested earlier in the digestive system, fermentation of sugars, production of vitamin like biotin, vitamin K, mediation of immune responses, and protection of the host against invasions by alien microbes. Viruses and protozoa can also be a part of the gut micro flora, but these normally form only minor components of the total resident population of microorganisms in healthy individuals.

Non-pathogenic, pathogenic and potentially pathogenic microorganisms living in a state of equilibrium determined by their own ecosystem within the large intestine take part in the local immunological and metabolic processes as well as in those affecting the organism as a whole (Benno *et al.,* 2002).

In recent years the balance between the harmful bacteria and beneficial ones has been distributed. Illness, dietary changes, stress, ageing, food poisoning and the use of medications can contribute to this imbalance. The indiscriminate use of antibiotics is particularly detrimental to the gut flora since they are unable to distinguish between beneficial and harmful bacteria. They can wipe out the beneficial bacteria which leaves the bowel vulnerable for invasion by potentially dangerous bacteria. This can lead to side effects such as diarrhea, inflammatory bowel disease colon cancer and other gastrointestinal disorder (Shanahan, 2002).

One way of maintaining a balance of the gut micro flora is to consume a diet supplemented with beneficial bacteria. For thousands of years microbial cultures have been used in the 19^{th} century with the aim of preventing and curing diseases. However it was only at the beginning of this century that this practice was first put onto a scientific basis by the work of Metchnikoff at the Pasteur institute in Paris. He observed longevity in Bulgarian peasants and associated this with their elevated intake of soured milks what is now known as probiotics.

Probiotics are bacteria which when consumed in certain amounts confer health benefits beyond inherent nutrition. These probiotics work to repopulate internal environment and help support normal intestinal balance. The best known probiotics are the Lactic Acid Bacteria (LAB) especially the *Lactobacillus sp.* and *Bifidobacterium sp.* other bacterial species like *Escherichia coli, Streptococcus sp., Enterococcus sp.* and Bacteroides and some fungal species are also been proven to be used as probiotics. A number of potential benefits arising through the consumption of probiotics include prevention of colon cancer (Guarner and Malagelada, 2003), alleviation of

lactose intolerance (Gilliland, 1990), reduction of cholesterol levels, lowering of blood pressure, improvement of immune function, increased resistance to infectious diseases, particularly of the intestine and reduction in allergy. Possible health benefits include balance of pH, prevention and treatment of diseases like acute diarrhea, inflammatory bowel diseases and other GI disorders.

Oral consumption of health-promoting lactic acid bacteria or probiotic has been associated with the prevention, alleviation, or cure of diverse intestinal disorders such as viral and bacterial diarrhea, adverse effects of abdominal radiotherapy, constipation, inflammatory bowel disease, and food allergy (Gilliland, 1990; Hammes and Tichaczek, 1994; Salminen *et al.*, 1996). Much of the early evidence on the actual health effects of probiotics was anecdotal, but during the last few years data based on rigorous clinical studies indicating real health-promoting properties of certain well-characterized strains have started to accumulate (Lee and Salminen, 1995).

Commercial products contain probiotic strains like Lactobacillus, Bifidobacterium and certain species of Streptococcus. Lactic acid bacteria are widely used as probiotics to confer health benefits to the consumer. Lactic acid bacteria are known to be of practical importance. One of the most important applications of Lactic acid Bacteria (LAB) is its use as probiotics. From all LAB types, Lactobacillus has some useful characteristics which make it useful for indusrial applications (Stiles, 1996). In order to influence human health, the lactic acid bacteria must survive passage through the upper regions of Gastrointestinal (GI) tract and persist in the colon. There must neither be any adverse immune response to the bacteria nor must they be affected by metabolic end products. The probiotics should be antagonistic to mutagenic or pathogenic organisms in the gut. They must be genetically stable and remain viable in the final food product.

Some limitations of the use of dairy products to deliver probiotics are the presence of allergens and requirement of cold environments. This fact has led to the launch of new products based on non-dairy matrices. Some claims related to probiotic products are lactose intolerance and fat content. Some

matrices have been used in the development of non-dairy probiotic products such as fruits, vegetables, legumes and cereals. Fruits and vegetables can be considered good matrices since they contain nutrients such as minerals, vitamins, dietary fibres, and antioxidants. The development of different probiotic fruit juices has been studied (*176,177*). Prado *et al.* (*178*) described a revision about a variety of non-dairy probiotic beverages.

It has been suggested that fruit juices could serve as suitable media for cultivating probiotic bacteria (Mattila-Sandholm *et al.*, 2002). Fruit juices have an established market sector as functional drink through sale of calcium- and vitamin-fortified juices, and they are consumed regularly, which is essential if the full benefits attributed to probiotics are to be experienced (Sheehan *et al.*, 2007).

Different studies have been carried out to explore the suitability of fruit juices such as tomato, beet and cabbage juices as raw materials for the production of probiotic drinks. *L. plantarum, L. acidophilus* and *L. casei* have been employed as probiotic bacteria cultures. Results have indicated that all the strains are capable of growth in the fruit juices mentioned and as a result, the microbial population increases significantly after 48 h of fermentation. Moreover, *L. plantarum, L. acidofilus* and *L. delbruekii* have shown to be resistant to the high acidic and low pH conditions during storage periods at 4°C. However, results on *L. casei* have indicated that this strain loses its viability during cold storage (Yoon *et al.*, 2004, 2005, 2006). Enrichment of the fruit juice-based medium with nutritive substances has also been studied.

Beverages of a new generation are those obtained by means of a controlled fermentation process performed by selected bacterial strains derived from the human digestive tract and manifesting probiotic characteristics, primarily from the genera *Lactobacillus*, *Bifidobacterium* and *Enterococcus*. The probiotic species representatives include *Lactobacillus acidophilus*, *Lb. johnsonii*, *Lb. casei*, *Lb. gasseri*, *Lb. plantarum*, *Lb. rhamnosus*, *Bifidobacterium longum*, *B. breve*, *B. bifidum*, *B. infantis*, and *Enterococcus*

faecium (Kaur IP *et al.,* 2002). The consumption of probiotic beverages increases the number of lactic acid bacteria in the large intestine. Lactic acid bacteria are capable of inhibiting the growth of many pathogens, such as *Escherichia coli*, *Salmonella* sp. (Zamfir M *et al.,*1999) , *Staphylococcus aureus* and enterococci frequently present in fermented food (Bengmark S, 2000). In addition, they are capable of inhibiting the growth of rotaviruses (Kaur IP *et al.,* 2002) that cause acute diarrhoea in children. In recent years, a positive effect of probiotic bacteria on the human immunological system, when used in children with innate HIV, has been indicated (Rundles-Cunningham S *et al.,* 2000).

The most frequently manufactured beverages and preparations containing probiotic lactic acid bacteria are based on milk and its derivatives. However, such products might prove unsuitable for people with an intolerance of lactose or an allergy to milk proteins. Fermented vegetable products, e.g. vegetable and fruit juices, are an alternative for this group of consumers.

Literature review

CHAPTER 2

LITERATURE REVIEW

In recent years, there has been an increase in awareness among consumers about alternative therapies to antibiotics. In the present era of antibiotic-resistance, pathogens and other looming microbial threats, the value of prevention of the infection and disease are recognized (Isolauri *et al.,* 2001). Hence, there has been more emphasis on preventive methods for disease occurrence rather than curative measures. Currently, antibiotic therapy can result in an altered intestinal microbial balance causing several unpleasant side effects that can persist even after the cessation of the treatment. The condition of the gastrointestinal tract is essential for our well being. The microflora limits the ability of potential pathogens to infect. It boosts the body's ability to resist infection, prevents morbidity and decrease antibiotic use (the spread of antibiotic resistant pathogens). Exposure of the immune cells of the intestinal tract to the right types of microbes in infancy may be important for the prevention of allergy development later in life. In addition to being a serious threat to human health, resistance to antimicrobial agents is a significant economic threat as well. These facts therefore suggest that intervention at the level of intestinal microflora may be important to enhance and sustain human health. Therefore, people all over the world are now opting to prevent or reduce the risk of diseases as against treating diseases. With this view in mind, the World Health Organization (WHO) recommends global programs to reduce the use of antibiotics in animals, plants and fishes, for promoting livestock growth. In human medicine, WHO recommends a reduction in the intake of antibiotics and an increase in efforts to prevent the disease through immunization with existing vaccines, and through the development of newer and more effective and safer approaches.

One such newer approach is the use of probiotics. Probiotics may play a very important role in helping the body protect itself from infection, especially along the colonized mucosal surfaces of the gastrointestinal tract. The evidence in the role of probiotics in maintenance of health or prevention of the disease is mounting and is supported in some cases by the blinded, placebo controlled human trials.

The relationship between certain foods and health benefits has been investigated for many years. Development of foods that promote health and well-being is one of the key research priorities of food industry (Klaenhammer and Kullen, 1999). This trend has favored consumption of foods enriched with physiologically active components such as prebiotics, probiotics, vitamins, minerals, dietary fiber, fish oils, and plant sterol (Betoret *et al.*, 2003). Probiotics are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance (Fuller,1989). The majority of probiotics recommended are the species of *Lactobacillus* including *L. acidophilus, L. plantarum, L. casei* and *Streptococcus lactis*, etc (Sindhu and Khetarpaul,2001). Probiotication is one of the methods used to produce fermented functional foods. Addition of probiotics to food provides several health benefits including reduction in the level of serum cholesterol, improvement of gastrointestinal function, enhancement of immune system and reduction in risk of colon cancer (Berner and O Donnell, 1998; Saarela *et al.*,2002; McNaught and MacFie, 2001; Rafter, 2003). For health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^6 cfu/g of product (Shah, 2001).

Currently, probiotic products are usually marketed in the form of fermented milk and yogurt. However, lactose intolerance and the cholesterol content are two drawbacks related to their consumption. It has been suggested that fruit juice could serve as a good

medium for cultivating probiotics (Mattila-Sandholm, *et al.*, 2002). Fruits and vegetables are healthy foods, because they are rich in antioxidants, vitamins, dietary fibers and minerals. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow & Delahunty, 2004).

2.1 History

The history of probiotic bacteria and their beneficial properties of live microbial supplements such as fermented milks go back many centuries. The use of live bacteria for health benefits is not a new concept. They were used in the treatments of body ailments, which has been mentioned even in Biblical scriptures. Similarly in Ayurveda, one of the pioneering medical sciences that date back to around 2500 BC, the consumption of yogurt has scientists in the early ages as Hippocrates considered fermented milk not only as a food product in terms of nutrition but also as medicine.

Elie Metchnikoff is regarded as the pioneer in the development of modern day probiotics. As early as 1907 Metchnikoff stated in his book "The Prolongation of Life" that consumption of large quantities of cultured foods such as sour milk, butter milk and yoghurt containing the microorganism termed as Bulgarican bacillus helped to maintain good health and long life of pheasants. Metchnikoff suggested that the lactic acid bacteria in fermented milks eliminated putrefactive bacteria from the gastrointestinal tract, thereby laying the foundation of modern day probiotics. Later, the Bulgarican bacillus was renamed Lactobacillus bulgaricus and is now called L.*delbrueckii* sub sp. bulgaricus. Metchnikoff proposed that consumption of fermented milk would "seed" the intestine with harmless lactic-acid bacteria and decrease the intestinal pH and that this would suppress the growth of proteolytic bacteria.

Kollath first introduced the term "probiotics" in 1953 (Hamilton *et al.*, 2003). Probiotics are microbially derived factors that stimulate the growth of other microorganisms. Roy fuller later suggested a definition of probiotics that has been widely used: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance."

2.2 Definition

The World Health Organization defines a probiotic as "Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Bacteria that exert a beneficial effect in intestinal function and promote good health are therefore known as probiotic bacteria.

The three main aspects of the definition are:

- The micro-organisms (bacteria) are alive,
- The bacteria are administered orally,
- The bacteria should be capable of reaching the intestine alive, in order to have an influence on the microbial balance.

There are also situations where the cell viability is not required for probiotic activity such as improved digestion of lactose, immune system modulation and antihypertensive effects. In these situations, health beneficial effects have been linked to non-viable cells or to cell components, enzyme activities or fermentation products. There is also debate as to whether the concept of probiotic bacteria should include dead microorganisms or bacterial fragments. In this regard, the concepts of acid bacteria that have a capacity to interact with the host mucosa and may beneficially modulate the immune system independently of the viability of Lactic acid bacteria.

2.3 Description

In general, Lactobacilli are the largest genus of the lactic acid bacteria group and include over 50 species. Lactobacilli commonly inhabit the gastrointestinal (GI) tract, oral and vaginal regions of humans and animals.

2.3.1 **Family:** Lactic Acid Bacteria

The genera, Lactobacillus, Carnobacterium, Enterococcus and Sporolactobacillus can then be divided into species, subspecies, variants and strains. Early genus and species have different characteristics but they are generally chained cocci or rod shaped gram positive, non motile, non sporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism.

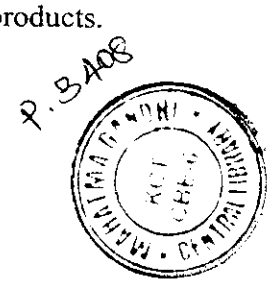
Lactic acid bacteria use lactose as their main source of carbon to produce energy. Lactic acid bacteria use energy to transfer lactose (main sugar of milk) through their cell membrane. The lactose is metabolized to lactic acid and in some species also acetic acid, ethanol and carbon dioxide. Lactic acid bacteria that only produce lactic acid as an end product are called homo fermentative; those that also produce acetic acid, ethanol and carbon dioxide are termed hetero fermentative. The by-product of this reaction is energy that the bacteria use for growth. The end products of fermentation ultimately change taste and texture of food.

2.3.2 **Genus:** Lactobacillus

There are many species of Lactobacillus bacteria that are found in a variety of environments, from dairy products to the human gastrointestinal tract (GI). They are either micro-aerophilic or anaerobic and strictly fermentative. The G+C content of their DNA is between 32 and 51 mol%. The optimum growth occurs within 35-40°C but it can tolerate temperatures as high as 45°C. The optimum pH for growth is between 5.0 and 5.6 and its acid tolerance is from 0.3-1.9% titrable acidity.

Species of Lactobacillus that have been isolated from the GI tract are, *L.acidophilus, L.fermentum, L.plantarum, L.brevis, L.caesi, L.leichmanii,* and *L. mintus.* Some of the most common Lactobacilli used for dietary intake are *L.acidophilus, L.casei, L.paracasei, L.plantarum* and *L.rhamnosus.*

Lactobacilli are able to live in highly acidic environments of pH 4-5 or lower. This pH is well below the pH of other lactic acid bacteria can live in and because of this; Lactobacillus is responsible for the final stages of fermentation in products.



2.3.3 Nutritional supplementation

Nowadays, several strains of probiotic bacteria are known, but their utilization is restricted by the fact that an effective probiotic is supposed to proceed along the acidic pH of the stomach and is able to resist the digestion of bile and pancreatic juice and finally sticks to the surface of some cells in the intestinal wall. The lifespan of the stuck probiotic is short, ranging from a few days to a few weeks and it usually lasts for a short period of time following its regular intake. Colonizing probiotics compete with other

microorganisms for nutrients and appropriate binding sites. Only probiotics capable of colonizing even if only temporarily, can exert the required immunological effect (Saxelin, 1996). The *in vitro* efficacy of the well-known probiotic strains has already been proved in clinical practice (O'Mahony *et al.*, 2001). The most widely used strains are those taken from sour dairy products and the intestinal system. The most frequently studied species include various species of Lactobacilli, Steptococcus, Bifidobacteria, *Saccharomyces boulardii,* but under certain conditions other strains in the intestinal microflora, such as *Escherichia coli.*, can also be used as probiotics (Floch, 2003 and Kruis *et al.*, 2004).

In order to become suitable for producing health improving foods, namely functional foods, individual probiotic strains need to meet certain requirements (Ouwehand and Salminen, 1999 and Saxelin *et al.*, 1999). The following criteria are listed for effective probiotic bacteria (Salminen *et al.*, 1996 and Salminen *et al.*, 1999).

- Maintenance of the biotic potential.
- Good taste and flavor following fermentation.
- Mild acidity in the course of storage.
- Preservation of the capability of colonizing in the course of food technology and storage.
- High degree of stability during storage.
- Stability in the course of freeze-drying or other drying procedures.
- Accurate and reliable determination of the strain.
- The dose-dependent effects.

Lactic bacteria supplements are available in different forms, including tablets, capsules and freeze-dried preparations. It is estimated that a dose of at least 108 (one billion) of live bacteria delivered to the appropriate site.

2.3.4 Significance

Lactobacilli have many important roles in industry. They contribute to the production of some chesses, yogurt and other products. The lactic acid produced by Lactobacilli inhibits the growth of other organisms and lowers the pH of the product in these products. The starter cultures for such products are carefully cultivated and maintained because their metabolic end products contribute to the flavor of the final food product. Additionally, some of the Lactobacilli's, metabolic reactions are intentionally manipulated to breakdown milk proteins during cheese production.Lactobacillus have been examined for their effectiveness in the prevention and treatment of a diverse spectrum of gastrointestinal disorders such as antibiotic-associated diarrhea (including *Clostridium difficile* associated intestinal diseases), infectious bacterial and viral diarrhea (including diarrhea caused by rotavirus, *Shigella, Salmonella,* enterotoxigenic *E.coli, Vibrio cholerae* and human immunodeficiency virus/ acquired immunodeficiency disorder, enteral feeding diarrhea, *Helicobacter pylori* gastroenteritis, sucrase maltase deficiency, inflammatory bowel disease, irritable bowel syndrome, small bowel bacterial overgrowth and lactose intolerance.

A very modern issue of LAB is there use as beneficial live components in so-called "healthy eating". The central aim behind this idea is to modulate the human gut microflora by administering health–relevant bacteria or by stimulating their growth by providing specific substrates of oligo- and polysaccharidic nature which cause an increase of their population.

Interaction of host/ microorganisms occurs by enzyme production which helps digestion (lactase), diminish production of ammonia, amines or toxic enzymes and favour a good functioning of the intestinal mucosa (Gregor *et al.,* 2003).

Lactobacillus has been found to inhibit intestinal bacterial enzymes involved in the synthesis of colonic carcinogens. It can help the body by protecting against colon cancer and the adverse effects of chemotherapy and radiotherapy. There are many mechanisms by which probiotics enhance intestinal health, including stimulation of immunity, competition for limited nutrients, inhibition of epithelial and mucosal adherence, inhibition of epithelial invasion and production of antimicrobial substances. Furthermore, they can be taken as a preventive against food poisoning when traveling.

Lactic acid bacteria are an important barrier against pathogenic microorganisms passing through the digestive tract, preventing their colonization by blocking the specific sites and by consuming the nourishing substrate. The favorable microorganisms stabilize pH in the digestive tract, obstructing the development of pathogenic organism and favoring the growth of useful microorganisms (Ducluzeanu and Raibaud, 1989).

There are 2 ways to reduce the formation of pathogenic germs:

1. Production of substances with selective antibacterial effect (organic acids or hydrogen peroxide which are favorable).
2. Competition for the active sites on the digestive lumen walls (usually won by the organisms which are predominant).

2.3.5 Mechanisms of action

The mechanisms by which probiotics exert biological effects are still poorly understood, but the nonspecific terms such as colonization resistance or competitive exclusion are often used to explain their mode of action (S.Elo *et al.,* 1991). Colonization

resistance or competitive exclusion describes a phenomenon whereby the indigenous anaerobic flora limits the concentration of potentially pathogenic (mostly aerobic) flora in the digestive tract (E.J.Vollaard and H.A.L.Clasener,1994). The concept of competitive exclusion was first developed during the early 1970s when it was discovered that the administration of mixed adult intestinal microorganisms conferred adult-type resistance against *Salmonella* infection to newly hatched chicks (E.Nurmi *et al.*, 1992). Oelschlaeger (*2010*) reported that the effects of probiotics may be classified in three modes of action:

(i) Probiotics might be able to modulate the host's defences including the innate as well as the acquired immune system. This mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of (chronic) inflammation of the digestive tract or parts thereof. In addition, this probiotic action could be important for the eradication of neoplastic host cells;

(ii) Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones. This principle is in many cases of importance for the preven tion and therapy of infections and restoration of the microbial equilibrium in the gut;

(iii) Finally, probiotic effects may be based on actions affecting microbial products like toxins and host products, *e.g.* bile salts and food ingredients. Such actions may result in inactivation of toxins and detoxification of host and food components in the gut. The same author also stated that the kind of effect(s) a certain probiotic executes depends on its metabolic properties, the molecules presented at its surface or on the components secreted. Even integral parts of the bacterial cell such as DNA or peptidoglycan might be of importance for its probiotic effectiveness. The individual combination of such properties in a certain probiotic strain determines a specific probiotic action and as a consequence its effective application for the prevention and/or treatment of a certain disease.

2.3.6 Probiotics and gut disorder

The gastrointestinal tract is a complex ecosystem having diverse and highly evolved microbial community composed of hundreds of different microbial species. The interactions that occur between this complex microbial community and the human host have become the focus of scientific research due to increases in the incidence of illnesses associated with deficient or compromised microflora (e.g Gastrointestinal tract infections, inflammatory bowel disease (Crohn's disease and ulcerative colitis), irritable bowel syndrome, antibiotic-induced diarrhea, constipation, food allergies, cardiovascular disease and certain cancers).

Effective multidisciplinary research programs now complement conventional microbiology with molecular ecology techniques to provide culture-independent analysis of the gastrointestinal ecosystem.

Furthermore, as we acquire an understanding of gut microflora composition and processes such as intestinal adherence, colonization, translocation and immunomodulation, we are elucidating mechanisms by which these can be influenced. This knowledge not only allows scientists to define the activities and interactions of "functional food"- borne beneficial bacteria in the gut, but will also provide the scientific basis for the development of innovative biotechnology- based products tailored to prevent specific diseases and promote overall human gastrointestinal health.

Under normal conditions, the resident bacterial flora of the gut comprises up to more than 10^{12} cells per g dry weight faeces. It has been recognized that lactic acid bacteria and bifidobacteria play a major role in this complex environment since they may contribute considerably to the microbiological balance.

2.3.6.1 Probiotics enhance anti-infective defences in the GI tract

Several clinical studies have demonstrated the therapeutic and/or prophylactic efficacy or specific probiotics against acute viral gastroenteritis and antibiotic-associated diarrhea (including *Clostridium difficile* infection). Emerging evidence also suggests beneficial effects against *Helicobacter pylori* infection.

The evidence of efficacy against traveller's diarrhea remains, however, inconclusive. The precise mechanisms by which probiotics potentiate host gastrointestinal defenses and mediate protection are not fully known. There is evidence to suggest, however, that probiotics might contribute to host defense by reinforcing non-immunological defenses and stimulating both specific and non-specific host immune responses.

2.3.6.2 Antagonistic property of lactobacilli

The antagonistic property is attributed to the lowered pH, the undissociated acids and production of other primary and secondary antimicrobial metabolites produced by LAB. The metabolites produced by the fermentation process, except the volatile ones, are kept in the foods and result in growth inhibition of food spoilage or poisoning bacteria and detoxification of noxious compounds of plant origin. The primary antimicrobial effect exerted by LAB is the production of Lactic acid and reduction of pH. In addition, LAB produce various antimicrobial compounds, which can be classified as Low-Molecular-Mass (LMM) compounds such as hydrogen peroxide, carbon dioxide, diacetyl (2,3-utanedione), uncharacterized compounds, and High-Molecular-Mass (HMM) compounds like bacteriocin.

2.3.6.3 Lactose intolerance

There is convincing evidence from several studies that lactose intolerance symptoms reduce with the consumption of probiotic dairy products. The mechanism of action of probiotic bacteria and fermented product include lower lactose concentration in the product resulting in high lactase activity increasing the active lactase enzyme entering the small intestine. The bacterial enzyme, beta-galactosidase found in the ileum after consumption of fermented milk products with viable probiotic bacteria is the major factor that improves digestibility by lactose hydrolysis. Therefore, there is good scientific evidence on the alleviation of lactose intolerance symptoms by specific probiotic bacteria.

2.3.6.4 Rotavirus diahorrea

Several studies have shown selected probiotics such as *L.reuteri, L.casei, B.lactis* Bb12 and *L.delbrueckii* sub sp. *Bulgaricus* can shorten the duration of rotavirus diarrhea by approximately one day. Shortening of the duration of rotavirus diarrhea using the probiotic strain *L.rhamnosus* GG is perhaps the best documented probiotic effect. The mechanism behind this favourable outcome is associated with enhancement of IgA to rotavirus and serum IgA antibody level at convalescence. It is therefore suggested that certain strains of probiotic bacteria promote systematic and local immune response to rotavirus, which may be of importance for protective immunity against re-infection.

2.3.6.5 Allergy prevention and alleviation

The prevalence of allergic disease has been on the rise in the past decades and is likely to continue to do so. It has been found that differences in intestinal microbiota composition precede the development of some allergic diseases (Bjorksten *et al.*, 2001).

This therefore indicates potential application of probiotic bacteria in this area. Administration of *L.rhamnosus* GG and *B. lactis* Bb12 parentally to mother and during the first few months to infants with high risk of atopic disease, reduced the prevalence of atopic eczema (Isolauri *et al.*, 2001). Additionally, supplementation of extensively hydrolyzed whey formula with *L.rhamnosus* GG or *B.lactis* Bb12, has been found to be more effective than unsupplemented formula on eczema alleviation in infants with atopic eczema (Isolauri *et al.*, 2001).

2.3.7 The function of the food supplements in our body

1. Improve efficiency of our digestive system.
2. Manufacture vitamins, such as, biotin and vitamin K.
3. Produce antibacterial substances which kill or deactivate harmful bacteria.
4. Help maintain normal bacteria balance in lower intestines.
5. Kill yeast and fungus on contact.
6. May lower cholesterol.
7. May clear up skin problems.
8. May extend life span.
9. May enhance immunity.
10. May reduce symptoms from spastic colon.
11. May reduce diarrhea to long-term antibiotic use.

Research shows that stomach acid kills many Lactobacillus strains on contact which prevents those strains from reaching the intestinal tract. Also, many of the other strains that do survive the stomach acid do not adhere to the intestinal lining.

Even if there is scientific proof that a certain strain has probiotic characteristics, it does not mean that the strain is effective in every person, as each person has his/her own intestinal flora, which may limit the effectiveness of a probiotic product.

2.3.8 Functional foods

In the recent years, the stressful lifestyles, deterioration in personal health and lack of exercise has led to an increase in the consumer awareness about foods that are nutritional and have dietary benefits. The reduction on health care expenditures by government has also led to increased self medication and personal responsibility for health care. Research in nutrition has led to a number of discoveries on food ingredients that can be incorporated for health benefits. This education has made it challenging for the food industry globally to introduce food products with ingredients that are not only just nutritional but which combine taste and appearance with positive health benefits. Such foods are termed as functional foods. A functional food is a food or a food ingredient, not necessarily a nutrient, with a demonstrated health benefit, or with the capacity to protect against disease, beyond the fundamental nutritional attributes. Among the most promising targets for functional foods are the gastrointestinal functions including those that control of nutrient bioavailability. This can in turn modify the gastrointestinal immune activity. Some other functions are lipid homeostasis, which indirectly influence nutrient digestion or fermentation. The functional food market has also been successful in UK, Japan and Australia.

2.3.8.1 Prebiotics

The application of probiotics has been supplemented with the concept of prebiotics. A prebiotic is defined as a non-digestable food ingredient that beneficially affects the

host selectively stimulating the growth, activity, or both of one or a limited number of bacterial species already resident in the colon (Dimer and Gibson, 1998). The most commonly used prebiotics are carbohydrate substrates with the ability to promote the components of the normal intestinal microflora which may evince a health benefit to the host. However, prebiotics can also be non-absorbable substrates which stimulate the growth of probiotics. When the two are applied together the concept is defined as synbiotic. At present, most prebiotics are directed towards the growth of lactic acid bacteria due to their purposed health promoting properties.

The prebiotics identified as non-digestable carbohydrates include lactulose, inulin, resistant starch and a range of oligosaccharides that supply a source of fermentable carbohydrate for probiotic bacteria in the colon.

2.3.8.2 Synbiotics

The benefits of prebiotics on probiotics have given rise to the concept of synbiotics, in which probiotics and prebiotics are used in combination (Gibson and Roberfroid, 1995). The live microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for the growth (eg, a fructooligosaccharide in conjunction with a Bifidobacterial strain).

It has been claimed that this combination could substantially improve the survival of probiotic bacteria as well as offer the advantages of microecological balance of the gut microflora.

2.4 Cucumber

Cucumber (*Cucumis sativus L.*), a popular vegetable crop of the family Cucurbitaceae, is rich in phosphorus, potassium and oxalic acid and is popularly used in salads. Its seeds are diuretic, tonic and refrigerant. The odorous principle of Cucumis L.

is extractable with alcohol and is used in certain bouquet perfumes (Pandey, 2000). It is an annual trailing or climbing vine usually with flowers of both sexes on the same plant (monoecious).

Similar to watermelon, cucumbers are composed of mostly water, low in calories, and packed with nutrients.

The flesh inside the cucumber is dense and crunchy, but it is also the part mostly made of water. Its high water content helps keep the body hydrated, helps regulate body temperature, rids the body of excess fluids, and cleanses the body of harmful wastes and toxins.

2.4.1 Types of cucumber

2.4.1.1 Armenian cucumbers

Figure 2.1: Armenian cucumbers

Armenian cucumbers are long, thin, and slightly variegated in darker and lighter shades of light green or a yellowish-green. They have very soft seeds and a thin skin, requiring neither seeding nor peeling for eating.

2.4.1.2 English cucumbers

English cucumbers (a.k.a. hot house cucumbers) are long, thin, with a dark green skinThis cucumber has a mild, almost non-existent flavor, and is prized for its thin skin and minimal seeds.

2.4.1.3 Garden cucumbers

Figure 2.2: Garden cucumbers

These are the most common cucumbers in North America. They are relatively smooth skinned and dark green.

2.4.1.4 Kirby cucumbers

Kirby cucumbers are short and bumpy. They have a range of skin color from yellow to dark green.

2.4.1.5 Lemon cucumbers

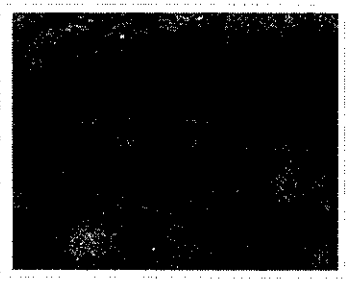


Figure 2.3: Lemon cucumbers

These cucumbers look like lemons. They are sweet, without that bitter edge that most cucumbers have, thin skins, minimal soft seeds, and flavorful.

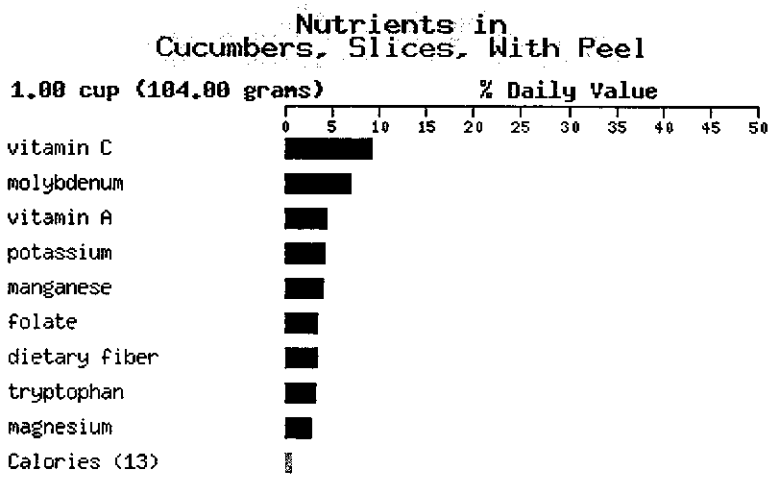
2.4.1.6 Persian cucumbers

Persian cucumbers are very similar to English cucumbers. They are shorter, with bumpy skin, but have a mild flavor and thin skin.

2.4.2 Components of cucumber

The flesh is a good source of ascorbic acid (vitamin C), caffeic acid, vitamin A, and folic acid.

The hard skin of the cucumber is rich in fiber and is an excellent source of a trace mineral, silica that helps strengthen connective tissue (including bone, cartilage, ligaments, tendons, and blood vessels). Potassium and magnesium are other important minerals in the skin of cucumbers.



2.4.3 Nutritional benefits

Cucumber has an impressive amount of water (about 96%) that is naturally distilled, which makes it superior to ordinary water. Its skin contains a high percentage of vitamin A, so should not be peeled off.

The cucumber contains alkaline-forming minerals and is an excellent source of vitamin C and A (anti-oxidants), folate, manganese, molybdenum, potassium, silica, sulfur, and lesser amounts of vitamin B complex, sodium, calcium, phosphorus and chlorine.

2.4.3.1 Heart health

When people participated in the DASH (Dietary Approaches to Stop Hypertension) Study, they added foods high in potassium, magnesium and fiber. Their blood pressure

dropped to healthier levels because of this. Those people in the study who ate a diet containing these compounds alongside other foods on this diet (low fat dairy foods, seafood, lean meat and poultry), they lowered their blood pressure by 5.5 points (systolic) over 3.0 points (diastolic). Also, in addition to this, Cucumber consumption may help reduce cholesterol.

2.4.3.2 Cancer prevention

Cucumber is Anti-Cancer in the way that it is high in Alkaline Minerals, for Cancer cannot live in an Alkaline Body. Cucumber's anticancer components include fisetin, lutein, caffeic acid and cucurbitacins. Although Cucumbers have been shown to have some antioxidant activities, these are low in comparison to many other, more nutrient packed vegetables. Cucumbers with more bitterness are usually rejected by the consumer, and yet, the bitterness is caused by a class of phytochemicals called cucurbitacins (oxygenated tetracyclic triterpenes), which have been shown to have some anticancer properties, but which also can be highly toxic if ingested. Fisetin, a flavonoid from the cucumber, has been shown to reduce the viability of prostate cancer cells in the laboratory without harming normal prostate cancer cells. Consumption of cucumbers has been found to be associated with lower risk of lung cancer among tin miners in China. Caffeic acid has been shown to have antiproliferative and apoptotic effects on human breast cancer cells in the laboratory. A Korean case-control study comparing the diets of breast cancer patients with a healthy control group of women found that the breast cancer patients consumed a significantly lower quantity of vegetables, including cucumbers, than the control group. A Greek study also found that women with breast cancer consumed significantly less cucumber than those without breast cancer. A Swiss study found that consumption of cucumbers, along with other fruits and vegetables, was connected to a significant protection against breast cancer.

2.4.3.3 Skin care

The silica in the cucumber is a vital component to healthy connective tissue, which includes muscles, tendons, ligaments, cartilage, and bone. Cucumber juice is usually recommended as a source of silica to improve the complexion and health of the skin, plus cucumber's high water content makes it naturally hydrating which is a key to glowing skin. Cucumbers are therefore often used topically for various types of skin problems, including swelling under the eyes and sunburn. Two compounds in cucumbers, ascorbic acid and caffeic acid, prevent water retention, which may explain why cucumbers applied topically are often very helpful for swollen eyes, burns and dermatitis, and are used to treat inflammatory skin conditions such as rosacea. The silica in cucumber is an essential component of connective tissue, and these includes muscles, tendons, ligaments, cartilage, and bone.

2.4.4 Health benefits

Many people are ignorant of the immense health benefits of cucumber and would avoid eating cucumber where possible. Fresh cucumber may taste "bland" to some but its thirst-quenching and cooling properties are refreshing. It acts as an anti-oxidant when taken together with fried and barbequed foods.

2.5 Pasteurization

Pasteurization is an age-long practice of subjecting beverages or drinks to high temperatures of below 100°C (60 – 90°) with the aim of destroying the vegetative cells and without altering the organoleptic quality of the food (Adams and Moss, 1997).

All hot-fill-hold and bottle pasteurization methods eliminated spoilage organisms that might decrease the shelf life, indicating that a short process at low temperature is sufficient to stabilize the cider. There were no significant differences between the treatments for alcohol, sugars, color, pH or titratable acidity. . Lower temperatures and shorter times resulted in best quality.

2.6 The importance of Non dairy products

Nondairy probiotic products have a big worldwide importance due to the ongoing trend of vegetarianism and to a high prevalence of lactose intolerance in many populations around the world. However, there is no question that the dairy sector, which is strongly linked to probiotics, is the largest functional food market, accounting for nearly 33% of the broad market, while cereal products have just over 22% (LFI 2006). A total of 78% of current probiotic sales in the world today are delivered through yogurt. Fruit juices, desserts, and cereal-based products featuring probiotics may be other suitable media for delivering probiotics (Cargill, 2009).

Application of probiotic cultures in nondairy products represents a great challenge. Probiotic viability in the food matrix depends on factors, such as pH, storage temperature, oxygen levels, and presence of competing microorganisms and inhibitors. It is important that the formulation maintains the activity and viability of the probiotic for extended periods of time (Shah, 2007). Since the probiotic cultures are included as ingredients to these kinds of products, they do not usually multiply, which sets great demands for the probiotic stability. Factors like water activity, oxygen tension, and temperature become increasingly important when dealing with these kinds of products. Storage at room temperature, which is common for many types of nondairy products,

such as cereal products, drinks, confectionary, and so on, can create an overwhelming challenge for probiotic stability (Matilla-Sandholm *et al.*, 2002).

Probiotics are living microbial supplements, which beneficially affect the host by controlling intestinal infection, controlling serum cholesterol levels, beneficially influencing the immune system, improving lactose utilization in lactose maldigestors, and having anticarcinogenic activity (Mc Naught and Mac Fie 2001; Saarela *et al.*, 2002; Rafter, 2003). Fruit juice is found as a healthy food product, and is currently consumed by a large percentage of the global consumer population (Verbeke, 2005). Fruits are healthy foods because they are rich in antioxidants, vitamins, dietary fiber and minerals. In addition, fruits do not contain any dairy allergens, such as casein and lactose. (Luckow and Delahunty, 2004a). Fruit juice was also suggested to serve as a good medium for probiotics (Tuorila and Cardello, 2002). Although cooperation of probiotics in fruit juices either by probiotication process, which allowed probiotic bacteria to grow in fruit juice, or direct addition provided the health benefit to consumers, the sensory quality of the product was not accepted (Krasaekoopt and Chea, 2007).

2.7 Dosage

Around the world there is increasing scrutiny of food and supplement claims. Both scientific and regulatory authorities are looking for assurance that a probiotic product can deliver live strains at sufficient concentration to the large intestine to provide a benefit to the individual. The current opinion is that levels of 10^6-10^7 cfu/g should be present at the time of consumption and thus 10^7-10^8 at time of manufacture for a claim, stated or implied, to be made. Recently some authorities have found lower levels present in trade samples.

OBJECTIVES

OBJECTIVE

1. To isolate Lactobacillus strain

2. To develop a probiotic juice

3. To evaluate various parameters during storage conditions

4. To carry out sensory evaluation.

Materials and methods

CHAPTER 3

MATERIALS AND METHODS

3.1 Sterilization of glass ware

The sterilization of glasswares such as sampling bottles, flasks, petridishes and test tubes after washing with detergent was carried out in an autoclave at 121°C for 15 minutes.

3.2 Isolation of lactic acid bacteria

Three different sources (idly batter, curd and fermented cucumber juice) were taken and microbial strain was isolated on MRS agar (Mann, de Ragosa, Sharpe, 1990).They were incubated for 18 h at 37° C. A single pure culture was isolated for further study.

Materials:

1. Idly batter
2. Fermented cucumber juice
3. Curd
4. Lactobacillus MRS agar
5. Petri plates
6. Conical flasks
7. Inoculation loop

Procedure:

1. 1 ml of each sample was taken and serially diluted.
2. The 4^{th} and 5^{th} dilutions were spread plated on MRS agar.
3. Plates were incubated overnight at 37 °C and observed for colonies.

4. From the MRS agar plates colonies were taken and Quadrant streaking was done to isolate individual colonies.

3.2.1 Isolation of pure culture

1. From the plates, single colonies with good zone of inhibition were selected.
2. The colonies were streaked in MRS slants.
3. The test tubes were incubated overnight at 37° C.
4. The slants were observed for growth.
5. These colonies were sub cultured and the original slants preserved for future use.

3.2.2 Characterization of *Lactobacillus*:

The samples were plated on MRS agar and the colonies were isolated. These colonies underwent preliminary screening by gram staining and the catalase test.

3.2.2.1 Gram staining

Gram staining is an empirical method of differentiating bacteria into two large groups based on differences in their cell walls. The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than gram-negative bacteria. Bacteria cell walls were stained by the crystals violet. Iodine was subsequently added as a mordant to form the crystal violet iodine complex so that the dye was not removed easily. However, subsequent treatment with ethanol dissolved the lipid layer from the gram-negative cells and enhanced the leaching of the primary stain from the cells. In contrast, the solvent dehydrated the thicker Gram-positive cell walls, closing the pores as the cell wall shrunk during dehydration. As a result, the diffusion of the violet-iodine complex was blocked, and the bacteria remained stained. Finally, a counter stain of safranin was applied to the smear to give decolorized gram-negative bacteria a pink colour.

Materials:

1. Microbial strains

2. Crystal violet
3. Gram's iodine
4. Safranin
5. Ethanol (95%)
6. Glass slides
7. Inoculation loop
8. Bunsen burner

Procedure:

1. A small drop of distilled water was placed on the surface of a clean glass slide.
2. Using a sterile loop, a thin smear of the culture was made on the slide.
3. The smear was flooded with crystal violet for 1 min after which the stain was washed off under running water.
4. The dye was fixed with Gram's Iodine and washed with water after one min.
5. The smear was decolorized using ethanol (95%) and washed under running water.
6. Finally, the smear was counter –stained with safranin for 30 sec and then washed with water.
7. The smear was air dried and visualized under 100X oil immersion microscopy.

3.2.2.2 Catalase test

The catalase test identifies organisms which produce the enzyme catalase; this enzyme converts hydrogen peroxide to oxygen gas and water. Catalase helps protect bacterial cells against hydrogen peroxide. Hydrogen peroxide is a highly reactive compound which damages cell components. It is sometimes formed when the electron transport chain is used to produce energy. When a catalase positive organism is exposed to hydrogen peroxide, the hydrogen peroxide will bubble.

Materials:

1. Hydrogen peroxide
2. Microbial strain

3. Glass slide
4. Inoculation loop

Procedure:

1. Lactobacilli colonies were air dried heat fixed on glass slide.
2. Crystal violet stain was added and washed with water after 1min.
3. Iodine solution (mordant) was added and washed with water.
4. Decoloriser (95% Ethanol) was added until no violet color remains.
5. Safranin was added & washed with water after 1min.

3.2.3 Selection of Lactobacilli for inoculation in the juice

Materials:

1. Isolated Lactobacillus slants
2. Lactobacillus MRS broth
3. Cucumber juice
4. pH meter
5. Nephelometer
6. Centrifuge

Procedure:

1. The IB2 10^{-4} and 10^{-5} cultures and J2 10^{-5} cultures were selected.
2. Loopful of organism was taken from the sub cultured slant and inoculated in the broth.
3. The tubes were incubated overnight at 37°C.
4. 1 ml of the broth was taken and centrifuged at 10000 rpm for 10 mins.
5. The supernatant was discarded and the pellets were washed.
6. The organism was inoculated in 10 ml of cucumber juice and incubated at 37°C for 48 h.
7. The pH and turbidity were noted before and after fermentation.

3.3 Preparation and Pasteurization of Cucumber juice

Materials:

1. Cucumber juice
2. Palm sugar
3. Fruit juicer
4. Glass bottles with cap
5. Hot water bath
6. Thermometer

Procedure:

1. The cucumbers were washed well and sliced.
2. The vegetable was juiced using a fruit juicer.
3. 75 ml of the juice was taken in each glass bottle.
4. The bottles were tightly sealed.
5. The bottles were placed in a water bath and the juice was pasteurized at 63°C for 30 mins (MR Fazeli *et al.*, 2007).

3.4 Inoculation of Lactobacilli in juice

Materials:

1. Lactobacillus culture
2. MRS agar
3. Distilled water
4. Pasteurized cucumber juice
5. Petriplate
6. Test tubes
7. L rod

8. Centrifuge

Procedure:

1. 1 ml of the culture was taken and was serially diluted.
2. The diluted sample was spread plated using an L rod.
3. The plate was incubated overnight at 37°C.
4. The number of colonies were counted.
5. 1 ml of the sample was taken and centrifuged.
6. The supernatant was discarded and the pellets were washed.
7. 10^5CFU/ml of the organism was taken and inoculated in the pasteurized juice (Yoon *et al.*, 2005).

3.5 Determination of pH

pH is the measurement of H^+ ion activity. It measures active acidity. pH may be determined by measuring the electrode potential between glass and reference electrodes; pH meter is standardised using standard pH buffers. Use homogenized sample for the determination of pH.

Materials:

1. pH meter and electrode
2. Standard buffers – pH 4.0 and pH 7.0
3. Sample
4. Control

Procedure:

Standardization of pH Meter:

1. Press **mode** to select pH.

2. Press **setup** twice and then **enter** to clear the existing standardization buffers.

3. Press **std** to access the Standardize screen. Immerse the electrode into pH buffer 4.0.

4. Press **std** again the initiate standardization. After the reading is stable, the meter will return to the measure screen. Remove electrode from buffer. Rinse off with DI water and blot dry with filter paper.

Repeat steps 2 and 3 with buffer 7.0 and sample.

3.6 Determination of turbidity

Turbidity is measured by nephelometry. This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity.

Materials:

1. Laboratory or process nephelometer consisting of a light source for illuminating the sample and one or more photoelectric detectors with a readout device to indicate intensity of light scattered at 90E to the path of incident light. An instrument designed to minimize stray light reaching the detector in the absence of turbidity and to be free from significant drift after a short warmup period is used. The sensitivity of the instrument should permit detecting turbidity differences of 0.02 NTU or less in the lowest range in waters having a turbidity of less than 1 NTU. Several ranges may be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities. Differences in instrument design will cause differences in measured values for turbidity even though the same suspension is used for calibration. To minimize such differences, the following design criteria are observed:

1) Light source— Tungsten-filament lamp operated at a color temperature between 2200 and 3000EK.

2) Distance traversed by incident light and scattered light within the sample tube — total not to exceed 10 cm.

3) Angle of light acceptance by detector— Centered at 90E to the incident light path and not to exceed "30E from 90E. The detector and filter system, if used, shall have a spectral peak response between 400 and 600 nm.

2. Sample cells: Sample cells or tubes of clear, colorless glass or plastic are used. The cells are kept scrupulously clean, both inside and out, and discard if scratched or etched. Tubes with sufficient extra length or with a protective case are used, so that they may be handled properly. The cells are filled with samples and standards that have been agitated thoroughly and sufficient time is allowed for bubbles to escape.

3. Control and sample

4. Distilled water

Procedure:

1. General measurement techniques:

Tubidity is measured immediately to prevent temperature changes and particle flocculation and sedimentation from changing sample characteristics. If flocculation is apparent, the aggregates are broken by agitation. Dilution should be avoided whenever possible. Particles suspended in the original sample may dissolve or otherwise change characteristics when the temperature changes or when the sample is diluted. Air or other entrained gases in the sample are removed before measurement. Air bubbles should not be removed by letting sample stand for a period of time because during standing, turbidity-causing particulates may settle and sample temperature may change. Both of these conditions alter sample turbidity, resulting in a non representative measurement.

Condensation may occur on the outside of surface of a sample cell when a cold sample is being measured in a warm, humid environment. This interferes with

turbidity measurement. All moisture from the outside of the sample cell should be removed before placing the cell in the instrument. If fogging recurs, sample should let to warm slightly by letting it stand at room temperature or by partially immersing it in a warm water bath for a short time. Samples should be well mixed again.

2. Measurement of turbidity:

1. Set '0' using distilled water as blank.
2. The control is gently agitated.
3. The control is poured into the cell after the air bubbles disappear.
4. The turbidity is read directly from the instrument display.
5. The steps 1-3 are repeated with the sample.
6. The readings are tabulated.

3.7 Determination of titratable acidity

OH^- from the base reacts with the H^+ in solution to form water, more H^+ will break loose from the undissociated portion of the acid to take its place. To measure the total acidity, also called base neutralising capacity (BNC) of a sample, it has to be titrated with a base. That is, a solution of a base whose concentration is known must be added to the sample slowly until the neutralisation is complete by measuring the volume of base added. It can be figure out the original concentration of acid.

Materials:

1. 0.1N Sodium Hydroxide

2. 1% Phenolphthalein

Procedure:

1. 1ml of sample was taken in a conical flask.

2. 5ml of water was added and few drops of phenolphthalein.

3. The burette was rinsed and filled with 0.1N NaOH and titrated with the contents of conical flask.

4. The end point was noted by the colour change to pale pink.

5. The titration was repeated until we got a concordant value.

3.8 Determination of calcium

Materials:

1. Inhibitor:

Dissolve 4.5g hydroxylamine hydrochloride in 100ml of 95% ethyl alcohol or isopropyl alcohol.

2. Murexide indicator:

Prepare a ground mixture of 200mg of murexide with 100g of solid NaCl.

3. Sodium hydroxide 2N:

Dissolve 80g NaOH and dilute to 1000ml.

4. Standard EDTA solution 0.01M:

Dissolve 3.723g EDTA sodium salt and dilute to 1000ml. Standardize against standard Ca solution, 1ml=1mg calcium carbonate.

5. Standard calcium solution:

Weigh accurately 1g AR grade Calcium carbonate and transfer to 250ml conical flask. Place a funnel and add HCl till $CaCO_3$ dissolves completely. Add 200ml distilled water and boil for 20-30 min to expel CO_2. Cool and add few drops of methyl red indicator. Add NH_4OH 3N dropwise till intermediate orange color develops. Dilute to 1000ml to obtain 1ml=1mg.

Procedure:

1. Take 25 or 50 ml sample in a conical flask.
2. Add 10 ml of NaOH to raise pH to 12.0
3. Add 1 ml of inhibitor to the sample.
4. Add a pinch of murexide indicator.
5. Titrate immediately with EDTA till pink color changes to purple.

Note the volume of EDTA required (A'). Run a reagent Blank. Note the ml of EDTA required and keep it aside to compare end points of sample titrations. Standardize the EDTA (0.1M) solution following the procedure of calcium hardness from 1 to 4 using standard calcium solution.

Calculation:

$$\text{Calcium hardness as } CaCO_3 \text{ (mg/ml)} = \frac{A' * D * 1000}{\text{ml sample}} \qquad (3.1)$$

where,

A'=Volume of EDTA used by sample

D = mg $CaCO_3$ equivalent to 1 ml EDTA.

3.9 Determination of ascorbic acid

Ascorbic acid otherwise known as vitamin C is an antiscorbutic. It is present in gooseberry, bitter gourd etc. in high amounts. Generally it is present in all fresh vegetables and fruits. It is a water soluble and heat-labile vitamin. The method described below is easy, rapid and a large number of samples can be analyzed in a short time.

Ascorbic acid reduces the 2,6-dichlorophenol indophenol dye to a colorless leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue colored compound, the end point is the appearance of pink color. The dye is pink colored in acid medium. Oxalic acid is used as the titrating medium.

Materials:

1. Oxalic Acid 4%.
2. Dye Solution: Weigh 42mg sodium bicarbonate into a small volume of distilled water. Dissolve 52mg 2,6-dichloro phenol indophenol in it and make up to 200mL with distilled water.
3. Stock Standard Solution: Dissolve 100mg ascorbic acid in 100mL of 4% oxalic acid solution in a standard flask (1mg/ml).
4. Working Standard: Dilute 10mL of the stock solution to 100mL with 4% oxalic acid. The concentration of working standard is 100µg/ml.

Procedure:

1. Pipette out 5ml of the working standard solution into a 100ml conical flask.
2. Add 10mL of 4% oxalic acid and titrate against the dye (V_1 ml). End point is the appearance of pink color which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.
3. Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to known volume (100ml) and centrifuge.
4. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V_2 ml).

Calculation:

Amount of ascorbic acid mg/100g sample

$$= \frac{0.5\text{mg}}{V_1\ \text{Ml}} \times \frac{V_2}{5\text{ml}} \times \frac{100\text{ml}}{\text{Wt. of the sample}} \times 100$$

(3.2)

3.10 Determination of total carbohydrates

Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into more simpler sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides. Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630nm.

Materials:

1. 2.5 N-HCl.
2. Anthrone Reagent: Dissolve 200mg anthrone in 100ml of ice cold 95% H_2SO_4. Prepare fresh before use.
3. Standard Glucose: Stock – Dissolve 100mg in 100ml water. Working standard – 10ml of stock diluted to 100ml with distilled water. Store refrigerated after adding a few drops of toluene.

Procedure:

1. Weigh 100mg of the sample into a boiling tube.
2. Hydrolyse by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature.
3. Neutralise it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100ml and centrifuge.
5. Collect the supernatant and take 0.5 and 1ml aliquots for analysis.
6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. '0' serves as blank.
7. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water.
8. Then add 4ml of anthrone reagent.
9. Heat for eight minutes in a boiling water bath.
10. Cool rapidly and read the green to dark green color at 630nm.
11. Draw a standard graph by plotting concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis.
12. From the graph calculate the amount of carbohydrate present in the sample tube.

Calculation:

$$\text{Amount of carbohydrate present in 100mg of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

(3.3)

3.11 Microbial analysis

Viable cell count (Cfu/ml) were determined by the standard plate method with lactobacillus MRS medium.

Materials:

1. Sample

2. MRS agar

4. Petriplates

5. Test tubes

Procedure:

1. The test tubes and the petriplates were sterilized by autoclaving at 121°C for 15 minutes.

2. MRS agar is prepared by adding 2% agar to MRS broth and autoclaving at 121°C for 15 minutes.

3. 15 – 20 ml of the agar is poured in each plate and the plates were cooled.

4. The sample was serially diluted to 10^{-4} concentration.

5. 1 ml of the serially diluted sample was taken and pour plated in the petriplate.

6. The plates were incubated overnight at 37°C.

7. The colonies were counted for every three days for a period of 15 days .

3.12 Sensory evaluation

The method used for sensory evaluation is the 9 point hedonic rating. Hedonic rating relates to pleasurable or unpleasurable experiences. The hedonic rating test is used to measure the consumer acceptability of food products. Some 1 to 4 samples are served to the panelist at one session. He is asked to rate the acceptability of the product on a scale, usually of 9 points, ranging from 'like extremely' to 'dislike extremely'. Scales with different ranges and other experience phrases could also be used. The results are analysed for preference with data from large untrained panels. Semi-trained panels in smaller number are used to screen a number of products for selecting a few for consumer preference studies.

Procedure:

The sample and the control were distributed among panel members and they were asked to rate them for various parameters such as colour, flavor, taste, mouth feel, overall acceptability. The following hedonic rating sheet was distributed among the panel members to rate the sample and control.

Specimen evaluation card

HEDONIC RATING SCALE

Name: ..... Date: .....

Product: .....

Taste the samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us.

	CODE	CODE	CODE
Like extremely	.....	.....	.....
Like very much	.....	.....	.....
Like moderately	.....	.....	.....
Like slightly	.....	.....	.....
Neither like nor dislike	.....	.....	.....
Dislike slightly	.....	.....	.....
Dislike moderately	.....	.....	.....
Dislike very much	.....	.....	.....
Dislike extremely	.....	.....	.....

Reasons

Signature

Results and Discussion

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Isolation of lactic acid bacteria

The three different sources, idly batter, fermented cucumber juice and curd dilutions were serially diluted to 10^{-4} and 10^{-5} dilutions and streaked on lactobacillus MRS agar. Growth of milky white lactobacillus colonies were identified on all the three sources.

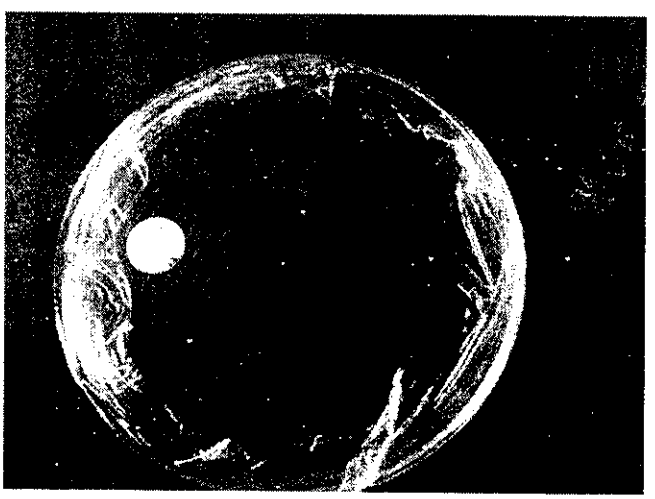


Figure 4.1: Isolated lactobacillus

4.2 Isolation of pure culture

Table 4.1: Isolation of pure culture

SOURCE	10^{-4}	10^{-5}
IB_1	+++	+++
IB_2	++++	++++
J_1	++	++
J_2	++	+++
C_1	+	+
C_2	+	+

Slant cultures were prepared from the quadrant streaked culture plates. The slants were observed for growth. The IB_2 10^{-4}, IB_2 10^{-5} and J_2 10^{-5} slant cultures showed good growth characteristics than the other cultures. They were selected for further analysis.

4.3 Chracterization of *Lactobacillus*

4.3.1 Gram staining

Morphologically different colonies on MRS agar plates were subjected to Gram staining. Most of the colonies were gram positive in nature, differing in morphology as rods or cocci. Several yeasts were also isolated. All gram-positive rod shaped bacteria were chosen for further characterization.

4.3.2 Catalase test

The catalase test was performed on the gram positive bacilli. Most strains were catalase negative while a few were catalase positive; the catalase positive bacteria converted hydrogen peroxide to water and oxygen gas resulting in the formation of bubbles. Since lactobacilli are known to be catalase negative bacteria, only the catalase negative bacilli were selected.

4.4 Selection of Lactobacilli for inoculation in the juice

Table 4.2: Selection of Lactobacilli for inoculation in the juice

SOURCE	0^{th} HOUR		7^{th} DAY	
	pH	TURBIDITY	pH	TURBIDITY
$J_2 10^{-4}$	6.91	53	4.26	51.3
$IB_2 10^{-4}$	7.05	50.2	4.29	63.0
$IB_2 10^{-5}$	6.69	57.1	4.20	88.1

The pH and turbidity at the 0^{th} hour and 7^{th} day were noted. There was reduction in pH and increase in turbidity in all the three samples. The Lactobacillus from idly batter showed good growth than the Lactobacillus isolated from fermented cucumber juice. The $IB_2\ 10^{-5}$ culture showed good reduction in pH from and increase in turbidity than the $IB_2 10^{-4}$. Hence it was selected for inoculating in the juice.

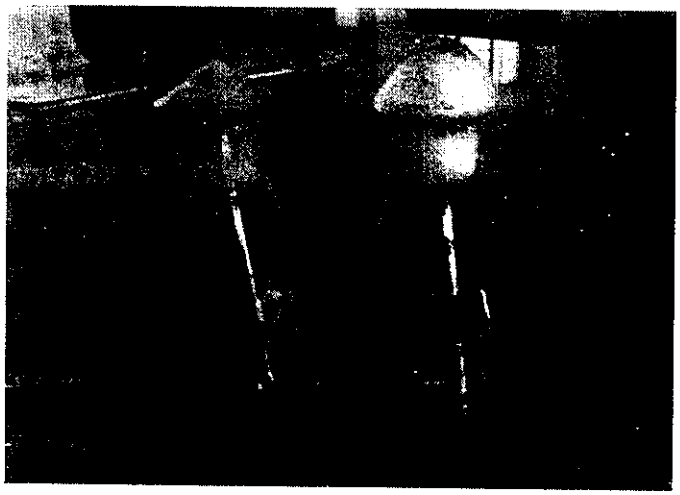


Figure 4.2: Lactobacilli from cucumber juice and idly batter inoculated in broth

4.5 Inoculation of Lactobacilli in juice

The plate was counted after overnight incubation at 37°C. It was found that 1 ml of the broth contain $>10^5$ colonies. 10^5 cfu should be added to 1 ml of the juice. $65x10^5$ cfu should be added in 65 ml of the juice. Therefore 65 ml of the broth was taken and centrifuged and the pellets were inoculated in each juice bottle containing 65 ml of pasteurized cucumber juice.

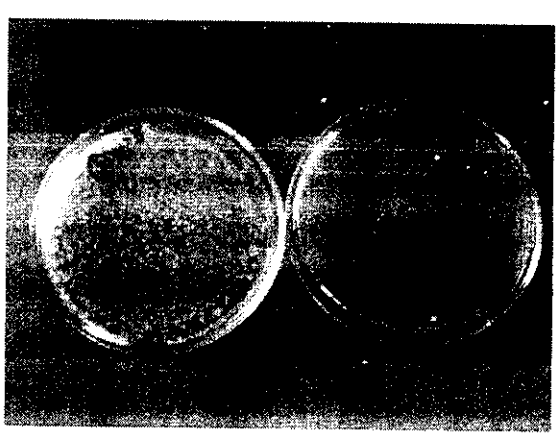


Figure 4.3: Lactobacilli colony

4.6 Determination of pH

Table 4.3: Determination of pH

S.N0	DAY	pH	
		CONTROL	SAMPLE
1	0	6.125	6.14
2	3	6.055	4.98
3	6	5.955	4:665
4	9	5.785	4.295
5	12	5.705	4.015
6	15	5.685	3.89

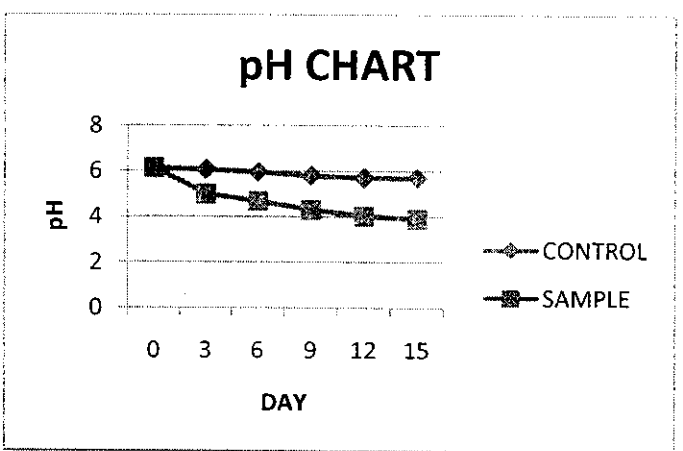


Figure 4.4: Comparison of pH between control and sample

pH of the pasteurized juice (control) and the pasteurized juice inoculated with LAB (sample) were determined once in three days for a period of 15 days. It was found that the pH decreased to acidic condition due to the production of lactic acid after fermentation and the pH decreased during the subsequent storage period in the sample containing lactobacillus. The pH of the control decreased from 6.15 to 5.70 and the pH of the sample decreased to 3.91 from an initial pH of 6.15.

4.7 Determination of Turbidity

Table 4.4: Determination of Turbidity

S.N0	DAY	TURBIDITY	
		CONTROL	SAMPLE
1	0	1295.5	1443
2	3	1368.5	1870.5
3	6	1363	1976.5
4	9	1388.5	2448.5
5	12	1388	2852
6	15	1376	3261

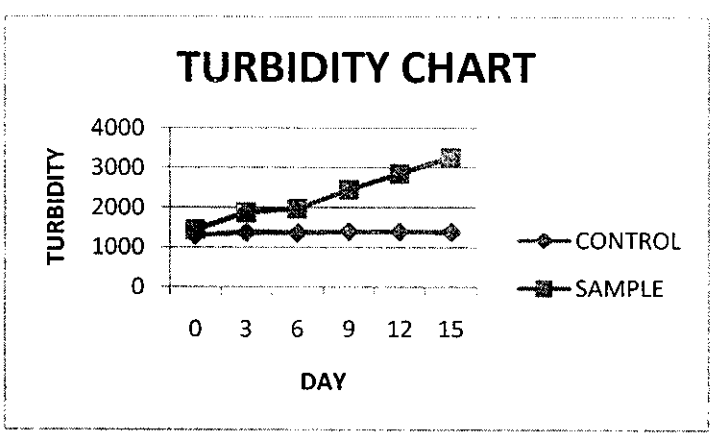


Figure 4.5: Comparison of turbidity between control and sample

Turbidity of the pasteurized juice (control) and the pasteurized juice inoculated with LAB (sample) were determined once in 3 days for a period of 15 days. It was found that the turbidity increased further after fermentation and during the subsequent storage period in the sample showing the increase in cell density of the lactobacillus. The turbidity of the control remained constant and the turbidity of the sample increased from 1445 to 3696.

4.8 Determination of acidity

Table 4.5: Determination of acidity

S.N0	DAY	ACIDITY	
		CONTROL	SAMPLE
1	0	2.14%	2.135%
2	3	2.125%	2.515%
3	6	2.145%	2.8%
4	9	2.135%	2.805%
5	12	2.14%	3.13%
6	15	2.11%	3.45%

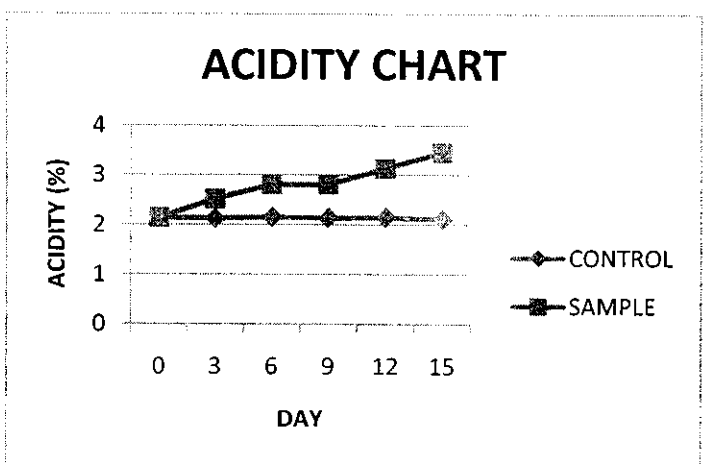


Figure 4.6: Comparison of acidity between control and sample

Acidity of the pasteurized juice (control) and the pasteurized juice inoculated with LAB (sample) were determined once in 3 days for a period of 15 days. The acidity of the control remained constant and the acidity in the sample increased sharply due to the lactic acid production from an initial 2.14% to 3.45%.

4.9 Determination of Calcium

Table 4.6: Determination of Calcium

S.N0	DAY	CALCIUM	
		CONTROL (mg)	SAMPLE (mg)
1	0	10	9.5
2	3	10	8
3	6	9.5	8.5
4	9	9	9
5	12	9.5	9.5
6	15	9.5	8

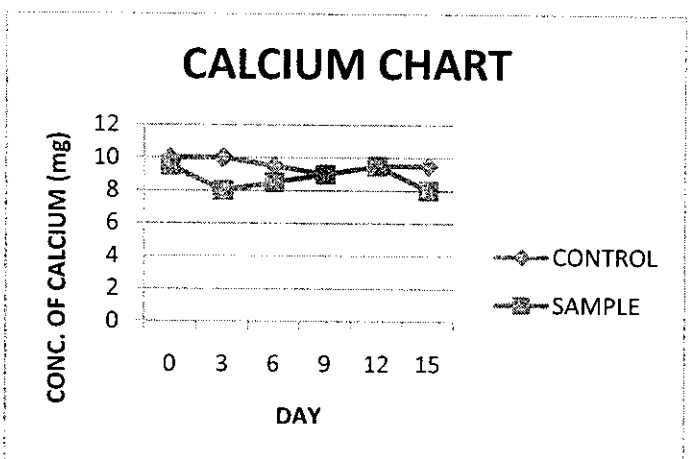


Figure 4.7: Comparison of calcium between control and sample

Calcium content of the pasteurized juice (control) and the pasteurized juice inoculated with LAB (sample) were determined once in 3 days for a period of 15 days. It was found that the calcium content remained constant before fermentation and during the storage period in both the control and the sample.

10 Determination of Ascorbic acid

Table 4.7: Determination of Ascorbic acid

S.N0	DAY	VITAMIN C	
		CONTROL (mg/g)	SAMPLE (mg/g)
1	0	0.02	0.025
2	3	0.02	0.02
3	6	0.02	0.02
4	9	0.02	0.02
5	12	0.02	0.02
6	15	0.02	0.02

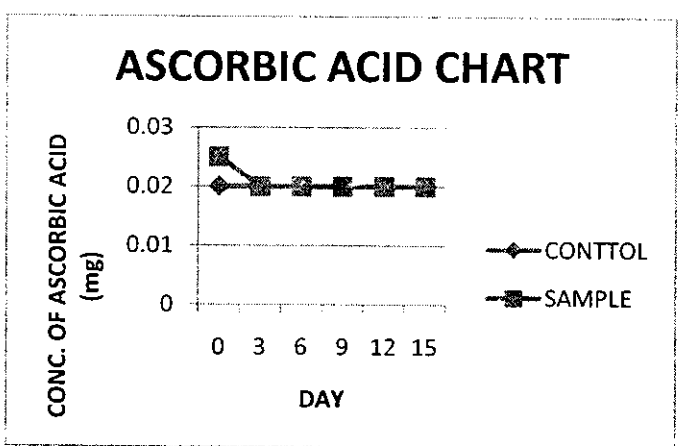


Figure 4.8: Comparison of ascorbic acid between control and sample

Ascorbic acid of the pasteurized juice (control) and the pasteurized juice inoculated with LAB (sample) were determined once in 3 days for a period of 15 days. It was found that the ascorbic acid content remained constant before fermentation and during the storage period in both the control and the sample.

4.11 Determination of total carbohydrate

Total sugar of the pasteurized juice (control) and the pasteurized juice inoculated with LAB (sample) were determined once in 3 days for a period of 15 days. The green colour formed was colorimetrically measured at 630 nm. It was found that the total sugar content remained constant before fermentation and during the storage period in the control. The amount of sugar was found to decrease sharply in the sample indicating the consumption of sugar by the lactic acid bacteria present in the sample. The amount of sugar initially present in the sample was 82.06 µg/ml which decreased to 47.28 µg/ml after the storage period.

Table 4.8a: Determination of total carbohydrate

S.NO	REAGENTS	BLANK	S_1	S_2	S_3	S_4	S_5	TEST
1	Vol. of standard (ml)	-	0.2	0.4	0.6	0.8	1.0	-
2	Conc. of standard (µg/ml)	-	20	40	60	80	100	-
3	Vol. Of sample (ml)	-	-	-	-	-	-	0.1
4	Vol. Of distilled water (ml)	-	0.8	0.6	0.4	0.2	-	0.9
5	Vol. Of Anthrones reagent (ml)	4	4	4	4	4	4	4
Keep in boiling water bath for 8 minutes								
6	OD at 630 nm							

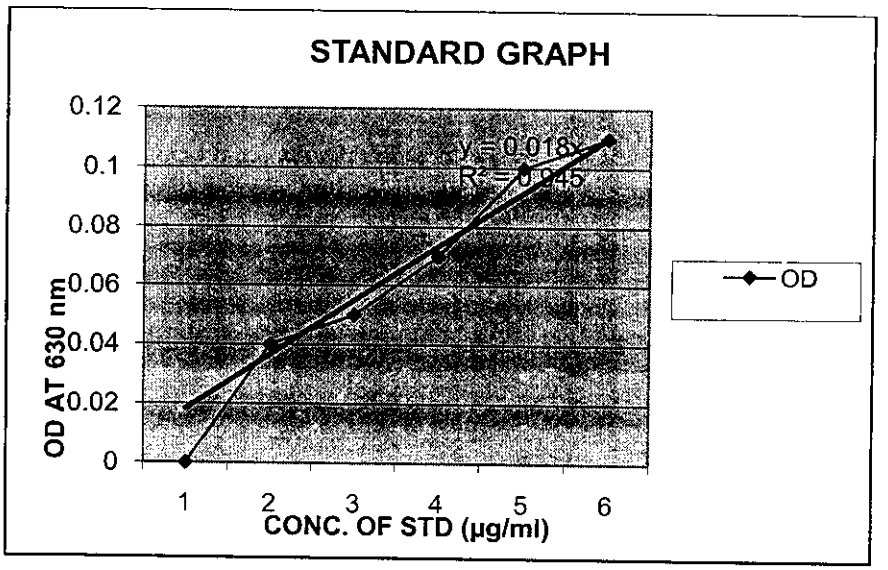


Figure 4.9: Standard calibration curve for total carbohydrate analysis

Table 4.8 b: Determination of total carbohydrate

S.NO	DAY	CONCENTRATION OF CONTROL (µg/ml)	CONCENTRATION OF SAMPLE (µg/ml)
1	0	82.085	82.1
2	3	81.535	71.19
3	6	80.43	67.4
4	9	81.26	53.115
5	12	81.055	49.78
6	15	81.495	47.325

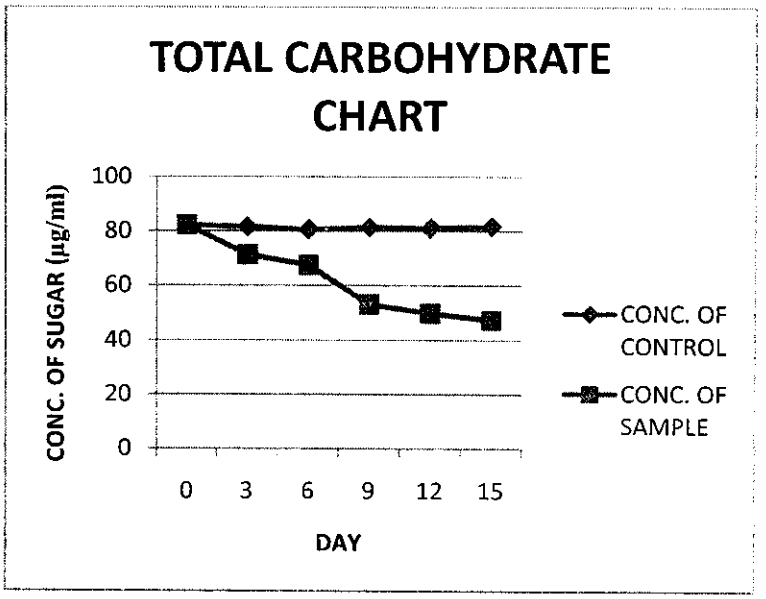


Figure 4.10: Comparison of total carbohydrate values between control and sample

4.12 Microbial analysis

The viable cell count (cfu/ml) was determined in the sample by standard plate count method. The sample was diluted to 10^{-4} dilution and plated in lactobacillus MRS medium. The numbers of colony forming units were counted once in three days for a period of 15 days. It was found that the microbial count increased from an initial count of 5×10^{5} to 2×10^{9} on the 12 th day. And then the population decreased to 4×10^{8} on the 15 th day.

Table 4.9: Microbial plate count

S.NO	DAY	CFU/ml
1	0	5×10^5
2	3	3×10^7
3	6	2×10^8
4	9	5×10^8
5	12	2×10^9
6	15	4×10^8

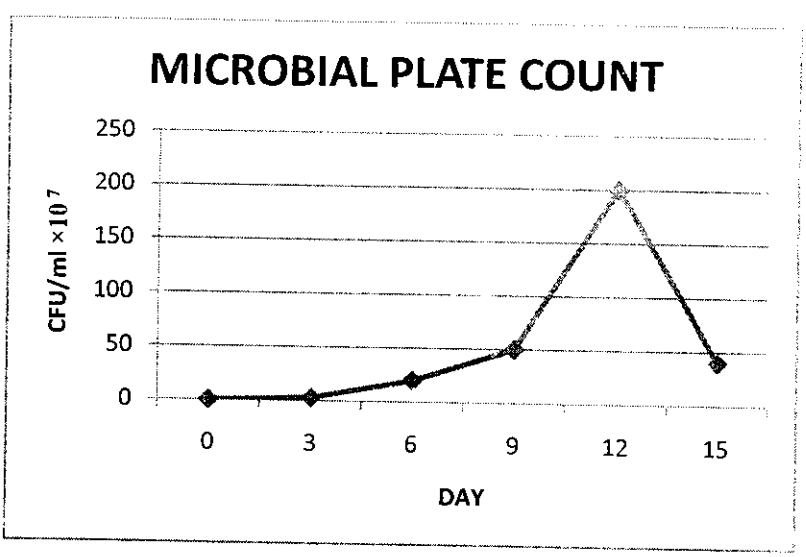


Figure 4.11: Microbial plate count

4.13 Sensory evaluation

Sensory parameters such as colour, flavour, taste, mouth feel and overall acceptability of the control and the sample inoculated with lactic acid bacteria were determined once in 3

days for a time period of 15 days. It was found that the control had a high rating of 8 when compared with the sample which had a rating of 7. The sample had higher rating for all other parameters such as flavour, taste, mouth feel and overall acceptability when compared to the control. The rating of flavour for sample was 8 and that of control was 7. The rating for taste of the sample was 7 and that of control was 6. The rating for mouthfeel of the sample was 8 and that of control was 7. The overall acceptability rating of the sample was 8 and that for the control was 7.

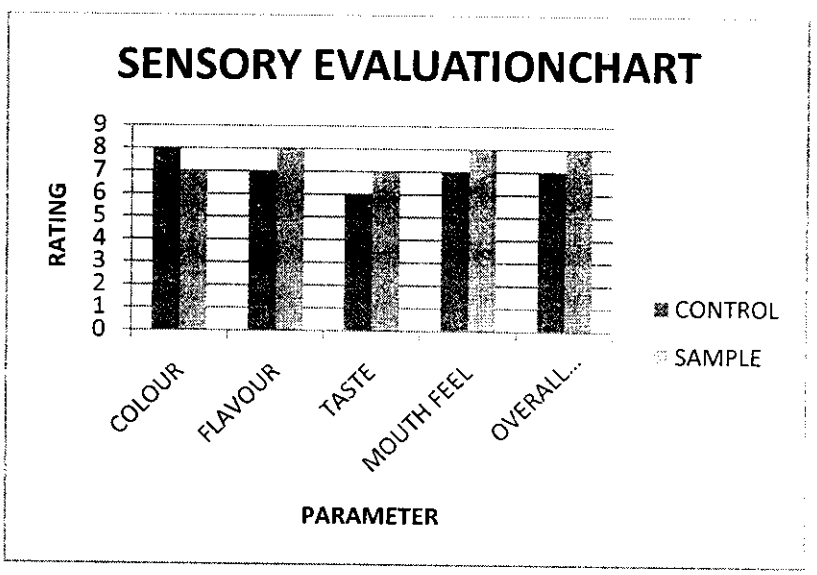


Figure 4.11: Comparison of sensory evaluation characteristics between control and sample

Conclusion

CHAPTER 5

CONCLUSION

The Lactic acid bacterial strain for inoculation in the fruit juice was isolated from idly batter. Juice was extracted from fresh garden cucumbers and palm sugar was added to taste. Pasteurization was carried out at 63°C for 30 mins and 10^5 cfu/ml of the isolated Lactic acid bacteria was added to the pasteurised juice. Fermentation was carried out at 37°C for 72 hours. The control and sample were stored at 4°C and the various parameters such as pH, turbidity, acidity, calcium, vitamin C and total sugar were evaluated during the storage period. The pH decreased sharply in the sample and remained unchanged in the control. The total sugar content was found to decrease gradually due to the consumption of sugars by the Lactic acid bacteria in the sample and they remained constant in the control. The turbidity increased in the sample indicating increase in the cell density and remained constant in the sample were lactic acid bacteria was not added. The acidity of the sample increased sharply in the sample and remained constant in the control. There was no change in the concentration of calcium and vitamin C in both the control and sample. Sensory evaluation tests were performed using 9 point hedonic scale which showed that the sample was more acceptable than control in terms of colour, flavour, mouth feel and overall acceptability. Microbial plate count was performed before and after fermentation and it was found that the population increased from 10^5 cfu/ml to 10^8 cfu/ml. Thus the probiotic cucumber juice was developed and the various parameters were evaluated during the storage period.

Appendix

CHAPTER 6

APPENDIX

Lactobacillus de Mann, Rogosa & Sharpe broth (g/1000ml)

Protease peptone	-	10g
Beef extract	-	10g
Yeast extract	-	5g
Dextrose	-	20g
Polysorbate 80	-	1g
Ammonium citrate	-	5g
Sodium acetate	-	5g
Magnesium sulphate	-	0.1g
Dipottasium phosphate	-	2g
Manganese sulphate	-	0.05g
pH	-	6.5±0.2

Lactobacillus de Mann, Rogosa & sharpe agar (g/1000ml)

To the components of *Lactobacillus* de Mann, Rogosa & Sharpe broth, add 12g of agar powder.

pH - 6.5± 0.2

Gram stain reagents:

Crystal violet stain (g/100ml):

Solution A:

Crystal violet - 2g

Ethanol (95%) - 20ml

Solution B:

Ammonium oxalate - 0.8g

Distilled water - 80 ml

Mix solutions A and B; store for 24 hours before use.

Gram's Iodine solution (g/300ml)

Iodine crystals - 1g

Potassium iodide - 2g

Distilled iodide - 300ml

Grind dry iodine crystals and potassium iodide thoroughly in a mortat, adding water (few ml at a time) and rinse into a new bottle with the rest of the water.

Safranin solution (g/100ml)

Stock safranin solution:

Safranin - 0.25g

Ethanol (95%) - 100ml

References

CHAPTER 7

REFERENCES

1. Adams, ME. And Moses, MO. (1999) 'Food microbiology', The Royal Society of chemistry, UK.
2. Bengmark, S. (2000) 'Colonic food: pre- and probiotics', Am J Gastroenterol, Vol. 95,No.1, pp.S5–S7.
3. Benno, Y. He, F. Hosoda. M. Hashimoto, H. Kojima, T. Yamazaki, K. Iino, H. Mykkanen, H and Salminen, S. (1996) 'Effect of Lactobacillus GG yoghurt on human intestinal microecology in Japanese subjects', Nutr Today, Vol.31, pp.9-11.
4. Berner, L. and ODonnell, J. (1998) 'Functional foods and health claims legislation: Applications to dairy foods', Int.Dairy J, Vol.8, pp.355-362.
5. Betoret, N. Puente, L. Diaz, MJ. Pagán, MJ. García, ML. Gras, J. Martínez-Monzó. Fito, P. (2003) 'Development of probiotic-enriched dried fruits by vacuum impregnation', J.Food Eng, Vol.56, pp.273-277.
6. Björkstén, B. Sepp, E. Julge, K. Voor, T. and Mikelsar, M. (2001) 'Allergy development and the intestinal microflora during the first year of life', Journal of Allergy and Clinical Immunology, Vol.108, No.4, pp.516-520.
7. Buttris, J. (1997) 'Nutritional properties of fermented milk products', International journal of Dairy Technology', Vol.50, No.1, pp.21-27.
8. Cargill. (2009) Cargill beverage concepts will address consumer demands for health, taste and texture at IFT 2008. Available from: http://www.cargill.com/news-center/news-releases/2008/NA3007612.jsp. Accessed Jul 20,2009.
9. Dimer, C. and Gibson, GR. (1998) 'An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future stratergies', Int Dairy J, Vol.8, pp.473-479.
10. Ducluzeanu, R. and Raibaud, P. (1989) 'Les interactions bact.riennes dans le tube digestif. Revue Scientifique et Technique de l', Office International des Epizooties, Vol.8, pp.291-311.
11. Elo, S. Saxelin, M. Salinen, S. (1991) 'Attachment of Lactobacillus casei strain GG to human colon carcinloma cell line Caco-2: Comparison with other dairy strains', Lett.Appl.Microbiol, Vol.13, pp.154-156.
12. FAO/WHO (2002) 'Guidelines for the evaluation of probiotics in food'. London, Ontario, Canada.
13. Floch, MH. (2003) 'Probiotics, Irritable Bowel Syndrome, and Inflammatory Bowel Disease', Curr Treat Options Gastroenterol, Vol. 6, pp. 283-288.
14. Fuller, R. (1989) 'Probiotics in man and animals', J.of Appl.Bacteriol, Vol.66, pp.365-378.
15. Gibson, G.R. and Roberfroid, M.B. (1995) 'Effect of probiotic food on human health', J.Nutr, Vol.125, pp.1401-1412.
16. Gilliland, S.E. (1990) 'Health and nutritional benefits from Lactic Acid Bacteria', FEMS Microbiol, Vol.87, pp.175-188.

17. Gregor Reid. Jana Jass, M. and Tom Sebulsky. (2003) 'Potential Uses of Probiotics in Clinical Practice', Clin. Microbiol, Vol. 16, pp.658-672.

18. Guarner, F. and Malagelada, JR. (2003) 'Gut flora in health and disease', The Lancet, Vol.361, pp.512-519.

19. Hamilton, HJ. Miller, JM. and Bolin, TD. (2003) 'The role of probiotics in the treatment and prevention of H.pylori infection', Int J Antimicro Agents, Vol.22, pp.360-366.

20. Hammes, W.P. and Tichaczek, P.S. (1994) 'The potential of Lactic Acid Bacteria for the production of safe and wholesome food', Z. Lebensm.- Unters- Forsch, Vol. 198, pp.193-201.

21. Isolauri, E. Sütas, Y and Kankaanpää, P. (2001) 'Probiotics: effects on immunity', Am J Clin Nutr, Vol.73, pp.S44- 50.

22. Kaur, IP. Chopra, K. Saini, A. (2002) 'Probiotics: potential pharmaceutical applications', Eur J Pharm Sci, Vol.15, pp.1–9.

23. Klaenhammer, TR. and Kullen, MJ. (1999) 'Selection and design of probiotics', Int.J.of Food Microbiol, Vol.50, pp.45-57.

24. Krasaekoopt, K. and Chea, P. (2007) 'Probiotication of fruit juices using *Lactobacillus casei*', Special project, Faculty of Biotechnology, Assumption University, Bangtok, Thailand.

25. Kruis, W. Fric, P. Pokrotnieks, J. Lukas, M. Fixa, B. Kascak, M. Kamm, MA. Weismueller, J. Beglinger, C. Stolte, M. Wolff, C. and Schulze, J. (2004) 'Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine', Gut, Vol. 53, pp.1617-1623.

26. Lee, Y.K. and Salminen,S. (1995) 'The coming age of probiotics', Trends Food Sci. Technol, Vol.6, pp.241-245.

27. [LFI] Leather Food Intl. (2006). Leatherhead Food International. The international market for functional foods. Functional Food Market Report.

28. Lidbeck, A. (1993) 'Lactobacilli and the normal human anaerobic microflora', Clin Infect Dis, Vol.16, pp.S181-S187.

29. Luckow, T. and Delahunty, C. (2004) 'Which juice is healthier? A consumer study of probiotic non-dairy juice drinks. Food Qual. Prefer: Available online at www.sciencedirect.com

30. Mattila-Sandholm, T. Myllarinen, P. Crittenden, R. Mogensen, G. Fonden, R.and Saarela, M. (2002) 'Technological challenges for future probiotic foods', Int Dairy J, Vol.12, pp.173–182.

31. Mc Naught, CE. and MacFie, J. (2001) 'Probiotics in clinical practice: a critical review oof the evidence', Nutrition research, Vol. 21, pp.343-53.

32. Nurmi, E. Nuotio, L. and Schneitz, C. (1992) 'The competitive exclusion concept: Development and future', Int.J.FoodMicrobiol, Vol.15, pp.237-240.

33. Oelschlaeger, TA. (2010) 'Mechanisms of probiotic actions- A review', Int.J.Med.Microbiol, vol.300, pp.57-62.

34. O'Mahony, L. and Feeney. M. O'Halloran, S. Murphy,L. Kiely, B. Fitzgibbon, J. Lee, G. O'Sullivan, G. Shanahan,F. and Collins, J.K. (2001) 'Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice', Aliment Pharmacol Ther, Vol.15, pp.1219-1225.

35. Ouwehand, A. and Salminen, S. (1999) 'The health effects of viable and non-viable cultured milk', Int Dairy J, Vol.8, pp.749-758.

36. Pandey, BP. (2000) 'Economic Botany', S. Chand & Co. Ltd, New Delhi.

37. Prado,FC. Parada,JL. Pandey,A. Soccol,CR. (2008) 'Trends in non-dairy probiotic beverages', Food Res. Int, Vol.41,pp.111–123.

38. Rafter, J. (2003) 'Probiotics and colon cancer', Best Practice & Research: Clinical Gastroenterology, Vol.17, No.5, pp.849-59.

39. Rundles-Cunningham,S. Ahrne, S. Bengmark, S. Johann-Liang, R. Marshall, F. Metacis, L. Califano ,C. Dunn ,AM. Grassey, C. Hinds, G. Cervia, J .(2000) 'Probiotics and immune response', Am J Gastroenterol, Vol. 95, No.1, pp.S22–S25.

40. Saarela, M. Lahteenaki, R. Crittenden, SS. and Mattila-Sandholm, T. (2002) 'Gut bacteria and health foods – the European perspective', International Journal of Food Microbiology, Vol.78, pp.99-117.

41. Salminen, S. Ouwehand, A. Benno, Y. and Lee, Y.K. (1999) 'Probiotics: How should they be defined?', Trends Food Sci Technol, Vol. 10, pp.107-110.

42. Salminen, S.J. and Tuomla, E.M. (1998) 'Adhesion of some probiotic and dairy Lactobacillus strains to caco-2 cell cultures', Int journal of food microbiology, Vol.41, pp.45-51.

43. Salminen, S. Laine, M. Von Wright, A. Vuopio-Varkila, J. Korhonen, T. and Mattila-Sandholm, T. (1996) 'Development of selection criteria for strains to assess their potential in functional foods: A Nordic and European approach', Biosci Microflora, Vol.2, pp.23-28.

44. Saxelin, M. Salminen, A. and Isolauri, E. (1999) 'Clinical efficacy of a human Lactobacillus strain as a probiotic in functional foods', The Royal Soc Chem, Vol.7, pp.67-70.

45. Saxelin, M. (1996) 'Colonization of the human gastrointestinal tract by probiotic bacteria', Nutr Today, Vol.31, pp.5-8.

46. Shah, NP. (2001) 'Functional Foods from probiotics and prebiotics', Food Technol, Vol.55, No.11, pp.46-53.

47. Shah, NP. (2007) 'Functional cultures and health benefits', Int Dairy J, Vol.17, pp.1262-77.

48. Shanahan, F. (2002) 'The host-microbe interface within the gut', Best Practice and Research Clinical Gastroenterology, Vol.16, No.6, pp.915-931.

49. Sheehan, VM. Ross, P. Fitzgerald, GF. (2007) 'Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices' , Innov Food Sci Emerg Technol , Vol. 8, pp.279–284.

50. Sindhu, SC. and Khetarpaul, N. (2001) 'Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and protein', J.of Food Comp. Anal, Vol.14, pp.601-609.

51. Soccol, CR. Prado, FC. Parada,JL. (2007) 'Technological process to produce a coconut fermented beverage with probiotic properties', *BR patent PI0703244-7* (in Portuguese).

52. Stiles, M.E. (1996) 'Biopreservation by Lactic Acid Bacteria', Kluwer Academic Publishers, Vol.63, pp.225-230.

53. Tuorila, H. and Cardello,AV. (2002) 'Consumer responses to an off-flavour in juice in the presence of specific health claims', Food Quality and Preferences, Vol.13, pp.561-69.

54. Verbeke, W. (2005) 'Consumer acceptance of functional foods: Socio-demographic cognitive and attitudinal determinants', Food Quality and Preferences, Vol.16, No.1, pp.45-57.

55. Vollard, EJ. Clasener, HAL.(1994) 'Colonization resistance', Antimicrob. Agents Chemother, Vol.38, pp.409-414.

56. Yoon, KY. Woodams, EE. Hang, YD. (2004) 'Probiotication of tomato juice by lactic acid bacteria', J Microbiol, Vol. 42, pp.315–318.

57. Yoon, KY. Woodams, EE. Hang, YD. (2005) 'Fermentation of beet juice by beneficial lactic acid bacteria', Lebensm Wiss Technol, Vol.38, pp.73–75.

58. Yoon, K., Woodams, EE. Hang, YD. (2006) 'Production of probiotic cabbage juice by lactic acid bacteria', Bioresour Technol, Vol.97, pp.1427–1430.

59. Yoon, KY. Woodams, EE. Hang, YD. (2004) 'Probiotication of tomato juice by lactic acid bacteria', J. Microbiol, Vol.4, pp.315–318.

60. Zamfir, M. Callewaert, R. Coernea, PC. Savu, L.Vatafu , J. De Vuyst, L .(1999) 'Purification and characterization of bacteriocin produced by *Lactobacillus acidophilus* IBB 801', J Appl Microbiol, Vol. 87, No.6, pp.923-925.