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Advanced Processing Techniques for Probiotic Fermented Vegetable Juice: Exploring its Impact on Gut Microbiota of Human

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Abstract: *In today's fast-paced society, many individuals neglect their health, particularly their digestive systems, due to hectic schedules and poor diets. Probiotics, beneficial bacteria known to enhance gut health, can support a healthy lifestyle and are increasingly being incorporated into functional foods. While dairy-based probiotic products have traditionally dominated the market, there is a growing interest in non-dairy alternatives such as fruit and vegetable juices. These juices are rich in bioactive components and are suitable for those who are lactose intolerant or prefer plant-based options. This study evaluates the development and analysis of a probiotic-enriched bitter gourd (Momordicacharantia Linn.) juice, focusing on sensory attributes, microbial viability, pH, total soluble solids (TSS), titratable acidity, protein, fat, ash, moisture, total solids, mineral content, carbohydrate, and energy content over a 3-day fermentation period and subsequent 21-day storage at 4ºC. Sensory analysis indicated that while fresh juice scored higher in color and texture, probiotic juice developed a favourable flavour over time. The overall acceptability between fresh and probiotic juices was comparable Microbiological analysis showed an increase in total viable counts from (105) CFU/ml on day 1 to (107) CFU/ml by day 3, maintaining stability during storage. The pH ranged from 4.3 to 5.5, indicating slight acidity, while TSS increased to 2 ºBrix. Protein, fat, ash, moisture, and total solids content all showed an upward trend during fermentation, suggesting significant biochemical changes. Mineral analysis revealed consistent levels of iron, sodium, potassium, and calcium. Carbohydrates and energy content increased progressively, enhancing the nutritional profile of the juice. After 21 days of storage, the probiotic juice maintained acceptable pH, TSS, titratable acidity, and viable cell counts, with sensory evaluations indicating sustained or improved flavor, color, aroma, and texture. The findings support the potential of probiotic bitter gourd juice as a functional beverage with health benefits and good consumer acceptability.*
The findings are anticipated to contribute to the development of healthy, non-dairy probiotic beverages, meeting consumer demand for dairy-free and vegan-friendly options, and promoting better digestive health through increased probiotic intake..

Keywords: Probiotics, ph, Fermentation, Temperature, Bitter guard, vegetable

I. INTRODUCTION

People these days are so busy with their everyday lives that they are unable to take care of their bodies and as a result of their hectic schedules and poor diets, their digestive systems are suffering. They lead unhealthy lives, which puts their bodies at risk for several ailments affecting the digestive system. Probiotics are something we have that can support a healthy lifestyle. Alternatively, as everyone knows, we eat every kind of fruit and vegetable. Therefore, we should consume probiotics that come from fruits or vegetables.

Nowadays, there is a growing trend among consumers to seek out "functional foods" that contain bioactive ingredients like fiber, oligosaccharides, or probiotic microbes. These foods may help prevent or lower the risk of food-borne disease. **(Gibson and Roberfroid, 1995)**

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Fruit and vegetable juices are drunk by people of all ages and are thought to be healthful because they contain a high concentration of bioactive components, and are currently receiving more attention. People who are intolerant to dairy products due to health reasons or other factors may choose to drink fruit or vegetable juices that have probiotics added**. (Antunes *et al.*, 2013)**

Dairy-based drinks have dominated the worldwide probiotic drink market up to this point. Still, the market for plant-based goods has been expanding more quickly in recent years, and probiotics in fruit and vegetable juices have become more and more popular. Growing knowledge of the allergic properties and high fat and cholesterol content of milk has caused people to favor healthier options. **(Vasudha and Mishra, 2013)**

Numerous studies indicate that taking probiotic supplements through food offers several health advantages, including boosted defenses against infection **(McNaught and MacFie,2001),** enhanced digestive efficiency **(Saarela *et al.*, 2002)**, decrease in blood cholesterol levels **(Anandharaj *et al.*, 2014)**, and reduce the chances of colon cancer.

The early-life development of the symbiotic relationship between humans and microbes is an intriguing and significant biological phenomenon. In humans, host physiology and metabolism are significantly influenced by the gut microbiota.**(Scholtens*et al.*, 2012)**.

Probiotics are advantageous bacteria that have the potential to treat the creatures that consume them. To get the health benefits of probiotics, a daily intake of at least 106–107 CFU/mL is advised. **(Martins *et al.*, 2016)**. When used to humans, they consist of a viable mono- or mixed culture of bacteria that enhances the natural gut microbiota positively. **(FAO/WHO, 2006)**

Gut microbiota refers to microorganisms that colonize the entire human digestive system.In the gastral intestinal tract (GIT), probiotics competitively suppress the growth and multiplication of pathogens such as Bacillus cereus, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, and Clostridium spp**. (Panghal *et al.*, 2018)**

Among the beneficial health claims of probiotics are the prevention of diarrhea, alleviation of gastric discomfort, relief of inflammation, and improvement of immunity.Drinks that have been prebiotically treated may have a higher market value since helpful bacteria are present. **(Mukisa*et al.*, 2017).** As the cell culture breaks down fermentable sugars to create byproducts like lactic acid, which has antibacterial characteristics, and alters the end product to have a tangy and sour taste, fermentation employing probiotic strains may improve the aroma and taste profile and increase the shelf-life**. (Huang *et al.*, 2015).**

Momordicacharantia Linn. (Karela), often known as Bitter Melon or Bitter Gourd, is a tropical and subtropical climber from the Cucurbitaceae family. It is extensively dispersed in China, Malaysia, India, and tropical Africa. The Latin word Momordica means "to bite" (referring to the leaf's jagged margins, which appear to have been bitten). All parts of the plant, including the fruit, taste bitter because they contain a bitter chemical called momordicin, which is thought to have stomachic properties. **(Gupta et al., 2011)**

Vegetable Juices with Probiotics

Eat your food, and let medicine be your food, as Hippocrates once said. The term "functional foods" has given fresh life to foods with medical significance in the modern day. One possible functional food is a probiotic**(Scheinbach, 1998).** Foods that combine traditional nutrition with one or more components that promote health are referred to as functional foods. Other names for functional foods include designer foods, therapeutic foods, medicinal foods, superfoods, and nutraceuticals. **(Soccol *et al.*, 2010)**

Recently, a wide range of basic ingredients have been thoroughly studied to find the right matrix to create novel functional foods that aren't dairy **(Vasudha and Mishra, 2013).** Cereals, legumes, fruits, and vegetables are matrices utilized in the production of non-dairy probiotic products. Since they include nutrients including vitamins, minerals, dietary fibers, and antioxidants, fruits and vegetables are regarded as excellent matrices**(Patel, 2017)**

Probiotic Microorganisms

Probiotic microorganisms are typically sold as dried or deep-frozen culture concentrates that can be used as food additives in homes or businesses. These can be taken as dietary supplements (items in powder, capsule, or tablet forms) or as food products (fermented or nonfermented). These bacteria also need to meet a few additional requirements specified by the European Union to be classified as probiotics**. (Becquet, 2003)**

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Health professionals are pushing probiotics—food that has been supplemented with living microbes—for their positive benefits on human health. Probiotics are important for a variety of metabolic and immunological processes, and they may also be able to significantly reduce the symptoms of infectious disorders in children. Numerous metabolic, lifestyle, and diet-related disorders, including obesity, endotoxemia, insulin resistance, type 2 diabetes (T2DM), metabolic syndrome (MetS), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), nonalcoholic fatty liver disease (NAFLD), gastrointestinal tract cancers, and more, have been linked to disrupted gut microbial balance. So far, more than 900 human trials and multiple review papers have been released regarding the beneficial effects of probiotics. The research was carried out using various **(Makinen *et al.*, 2012)**

To qualify for the intended application or purpose, a probiotic must meet the following conditions in order to be viable and functional.

- Probiotics need to be categorized as non-toxic and non-pathogenic in order to be used by humans.
- Probiotics need to have a high survival rate and be resistant to stomach acids and bile toxicity.
- High adhesion qualities and the capacity to colonize the urinary and gastrointestinal systems.
- It has to be long-term stable, viable, and survive both during food fermentation processes and storage or the product's shelf life.

Fruit and vegetable juice fermentation by probiotic microbes

In several nations, the use of lacto-juices, or fermented vegetable juices, has grown. The primary ingredients used to make lacto-juices include tomatoes, red beet, carrots, celery, and cabbage. **(Demir *et al.*, 2006)**

They can be made in one of the two methods listed below:

- Fermentation of juice or mash made from vegetables.
- Standard procedure for fermenting vegetables, followed by pressing the juice.

There are three methods for lactic fermentation in vegetable and fruit juices:

- Organic fermentation is caused by microorganisms in the environment.
- Starter culture fermentation
- Starter culture fermentation of materials that have been heated.

When lacto-juices are being produced, the squeezed juice can first be sterilized and then infected with a starter culture, primarily a LAB, at a concentration ranging from 2 x 10^5 to 5 x 10^6 CFU/ml**. (Panda and Ray, 2007)**

To ensure the fermentation of premium juices, commercial starter cultures such as *Lactobacillus plantarum, Lactobacillus bifidus, Lactobacillus brevis, Lactobacillus xylosus,*and *Lactobacillus bavaricus* must be used. The following lists the criteria that are used to judge whether a strain is suitable**. (McFeeters, 2004)**

- The capacity of a substrate to grow a starter culture.
- The rate and amount of lactic acid produced.
- pH changes
- The production of biogenic amines and a decrease in nitrate concentration
- The type of metabolism and the culture's capacity to produce the desired sensory qualities of fermented products.

Health Effects of Probiotic Cultures

Consuming probiotic cultures has been linked to several health benefits, including (1) a decrease in obesity and atopic dermatitis; (2) suppression of Helicobacter pylori infections; (3) amelioration of irritable bowel syndrome symptoms; (4) antimutagenic, anticarcinogenic, and antidiarrheal properties; (5) immune system stimulation; (6) a decrease in serum cholesterol; (7) an improvement in lactose metabolism; (8) antimicrobial activity; and (9) a decrease in gastrointestinal infections, among other benefits. **(Zoumpopoulou*et al.*, 2017).**

The primary modes of action of probiotics include competition for nutrients and adhesion sites, generation of antimicrobial metabolites, alterations in environmental circumstances, and modification of the host's immunological

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response. Thus, the advantages of probiotic cultures as well as potential methods of action will be discussed**. (Raman *et al.*,2016).**

Applications of Probiotics

Fruit and vegetable products containing probiotics as a starter culture

Starter culture was created as a result of research into the probiotic potential of fermented fruit and vegetables **(Xu et al.,2018).** Research has demonstrated that using commercial probiotic cultures in fruits and vegetables is successful. The majority of formulas have been designed to use probiotic starter cultures, which is a favored technological technique to reduce variance and guarantee productuniformity **(Penas, Martinez-Villaluenga, & Frias,2017).** Products containing a mixed or single culture of living microorganisms are provided in various formulations inside the food matrixes in the forms of juice, liquids, suspension, pills, capsules, powder, granules, sachets, chewable bars, and so forth due to the growing consumer interest in probiotics **(Kolacek *et al.*,2017).**

Biocontrol, preservation, and antagonistic probiotics

To keep fruits and vegetables from spoiling and from becoming infected, LAB has historically been used in their processing and preservation. **(Swain, Anandharaj, Ray, & Parveen Rani,2014).** When it comes to enhancing the microbiological safety and overall quality of fermented foods, spontaneous fruit and vegetable fermentation yields several advantageous chemicals that are worth investigating. Plant pathogens have been proven to be antagonistic towards certain probiotic isolates.

Consequences for Probiotic Survival in Juices

Probiotics ability to withstand harsh gastrointestinal circumstances and their concentration in food are what mostly determine their health benefits. Therefore, even if the survival of the probiotics depends on the strain **(Tripathi and Giri, 2014)**, The product should have at least 107 CFU/mL at the end of its shelf life, which roughly translates to 109 CFU per serving **(Nualkaekul and Charalampopoulos, 2001).**

Juices contain certain vital nutrients (antioxidants, dietary fibers, vitamins, and minerals), but certain significant variables may hinder the survival of probiotics in juices. And some of them are,

- **Food variables:** sodium chloride, bacteriocins, artificial flavoring and coloring compounds, pH, titratable acidity, molecular oxygen, water activity, and presence of salt and sugar.
- **Processing variables:** volume, oxygen levels, packing materials and storage techniques, heat treatment, incubation temperature, and cooling rate.
- **Microbiological variables:** rate and proportion of inoculation, strains of probiotics.

One of the most crucial elements influencing probiotic life is pH. Because juices have a high concentration of organic acids and a low pH, which enhances the concentration of undissociated form, we can assume that juices have both the inherent antibacterial properties of acids and an acidic environment. Generally resistant, lactobacilli can thrive in liquids with a pH of 3.7 to 4.3. However, bifidobacteria are less acid-resistant, and they cannot survive in environments lower than roughly 4.6 pH.

Major microorganisms used as a probiotic

Lactobacillus sp.

The lactic acid bacteria that ferment dairy milk have been the subject of much research, but the molecular mechanisms behind the fermentation of plant-based milks have received relatively less attention **(Hayes *et al.*, 2007; Mende *et al.*, 2016; Ji *et al.*, 2021)**.

A group of organisms known as lactic acid bacteria (LAB) are responsible for the centuries-old tradition of fermenting dairy-based meals **(Bernardeau *et al.*,2006)**. Foods that have undergone fermentation are said to have better flavor and texture. For example, the production of exopolysaccharides from sugars improves the texture of cheeses and yogurts **(Korcz and Varga, 2021)** whereas the breakdown of proteins into amino acids and then volatile organic compounds greatly contributes to the flavor of cheese **(Yvon and Rijnen, 2001; Smit *et al.*, 2005)**. By adding bioactive small molecules like peptides and, in certain situations, increasing vitamin content, fermentation also provides well-

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established health advantages **(Şanlier *et al.*, 2019)**. Furthermore, fermentation increases product shelf life because LAB acidify the medium and produce a less provide a favorable habitat for further microorganisms and generate a number of substances, including bacteriocins, hydrogen peroxide, and organic acids, which stop the growth of other organisms **(Saranraj *et al.*, 2013)**

Bifidobacterium Sp.

Vitamins including riboflavin, thiamine, vitamin B6, and vitamin K, as well as bioactive compounds like pyridoxine, niacin, and folic acid, can all be synthesized by bifidobacteria. Particularly high in free amino acids and vitamins is fermented milk that has been inoculated with bifidobacteria. Bifidobacteria are different from lactic acid bacteria in that they mostly create L(+)-lactic acid, which is easier for humans to consume. This makes them important for people with metabolic acidosis or babies. By assisting in the ionization of minerals, bifidobacteria in food items may help improve the bioavailability of certain minerals **(McCartney, 2003)**. Single bacterial strains, combinations with other probiotic microorganisms, encapsulated bacterial cells, and co-encapsulated cells with prebiotics are some examples of probiotic compositions incorporating *Bifidobacterium.* Typical *Bifidobacterium* species include Bifidum, Bifidum animalis, Bifidum adolescentis, Bifidum breve, Bifidum infantis, and Bifidum longum, are utilized in probiotic compositions and are known to colonize the human gut **(O'Callaghan & van Sinderen, 2016)**.

The genus Bifidobacterium is one of the obligate anaerobes that make up the bulk of bacteria that invade the gut. Probiotic agents have been urged to employ Bifidobacterium strains that have historically been used in fermented dairy products and that are 'Generally Recognized As Safe' **(Picard *et al.*, 2005)**. Numerous probiotic strains have demonstrated positive impacts on human health. This comprises a number of bifidobacteria species and strains that are used in commerce, some of which were very recently introduced.

Streptococcus (S. thermophilus)

S. thermophilus is a Gram-positive bacteria that belongs to the order *Lactobacillales,* family Streptococcaceae, and phylum Firmicutes. It belongs to the class of bacteria known as lactic acid bacteria, which includes a variety of species from different genera, including *Weissella, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leunostoc, Oenococcus, Pediococcus, Tetragenococcus, and Vagococcus***(Stiles ME *et al.*, 1997).***S. thermophilus is*** a popular dietary ingredient that may be found in a variety of fermented foods. *S. thermophilus* is closely related to Lactococcus lactis, but it is even more closely related to other streptococcal species including several pathogens **(Mitchell TJ *et al.*, 2003).** Because S. thermophilus promotes quick acidification, it is essential to the fermentation of milk. S. thermophilus is the main and fastest-producing species among LAB throughout the maturing phase of handmade Montasio cheese, according to research done by **(Marino, Maifreni, and Rondinini *et al.*,2003)**. They came to the conclusion that the metabolic characteristics of the strain, which are impacted by variables including lactose-galactose metabolism, the proteolytic system, and ureolytic activity, determine how quickly the strain acidifies. Studies by **(Masud, Sultana, and Shah *et al.*,1991)** and **(Reddy, Khan, and Purushothaman *et al.*,1994)** are among the ones that have documented the presence of S. thermophilus and its function in the generation of acid in dahi samples that were made in Indian subcontinent households. Together with lactic acid, S. thermophilus also generates trace quantities of formate, acetoin, diacetyl, acetaldehyde, and secondary byproducts according to **(Ott, Germond, and Chaintreau *et al.*,2000)**.

Dairy Based Products

A probiotic beverage made with diary involves enriching milk **(Benton *et al.*, 2006),** typically sourced from cows, but also potentially from goats, ship or water buffalo with probiotics **(Virtual Medical Centre 2020).** The standard production process includes pasteurisation, where the milk is heated to 71.7°C for 15 to 25 seconds, swiftly cooled, below 3°C to enhance self-life, and then aseptically inoculated with probiotic strains for fermentation. This method aims to deactivate spillage, micro, isms and enzymes, preserving nutritional contents. Conversely, non-probiotic drains, utilise substitute, fruits, vegetables and oatmeal **(Chaudhary *et al.*, 2019).** For nonsubstrates sterilisation through autoclaving at 1:201°C at 15 psi for 15 minutes is necessary before fermentation **(Gangwar *et al.*, 2018)**

Milk, being a nutrient, dense substrate, provides ample carbon (lactose), nitrogen from casein and whey and minerals like calcium, phosphorus, sodium and potassium to support probiotic growths **(Virtual Medical Centre 2020)**

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Milk contains minerals that are vital to human health and development, as well as to dairy-related activities including cheese-making and protein-salt interactions **(Franzoi *et al.*,2017).** Dairy products are a great source of calcium, protein, vitamin D, potassium, and phosphorus—all of which are essential for supporting good bone health **(Rizzoli *et al.*,2014)**

Allergens

In general, two primary milk proteins, namely casein and whey, are responsible for triggering allergic responses in humans. The acidification process of cow's milk results in two distinct fractions: Fraction 1, presenting as a solid coagulum known as casein, constitutes 80% of the total milk protein, while Fraction 2 appears as a liquid consistency called whey, contributing 20% to the overall milk protein content (**Arnberg *et al.*, 2012).** Allergenic components within the casein fraction include 32% aS1-casein, 28% B-casein, 10% aS2-casein, and 10% K-casein **(Schulmeister *et al.*, 2009).** On the other hand, the whey fraction contains allergens such as alactalbumin (5%) and B-lactoglobulin (10**%) (Wal *et al.*, 2004)** along with traces of immunoglobulins, bovine serum albumin, and lactoferrin **(Restani *et al.*, 2009).** Food safety worries are centered more and more on allergenic substances. Food intolerance, pharmacological reactions, and intoxication are not the same as immunological reactions to allergens. According to a UN research, the most common allergenic foods include fish, soybeans, cow's milk, chicken eggs, peanuts, crabs, tree nuts, and gluten-containing cereals. These foods are referred to as the "Big Eight**."(Prado, M. *et al.*,2018, Ross *et al.*,2018, GómezArribas*et al.*,2018).**

Lactose intolerance

Dairy probiotic products are derived from bovine milk, which contains lactose, a sugar component found in animal-based milk. The breakdown of this sugar in milk requires lactase enzymes, specifically beta-galactosidase. The absence of these enzymes leads to an inability to hydrolyze or break down lactose into its monosaccharide units, namely galactose and glucose. When undigested or non-hydrolyzed lactose reaches the large intestines, bacterial enzymes degrade it, resulting in osmotic diarrhea, gastrointestinal pain, and flatulence for individuals who are lactose intolerant **(Khan et al., 2021).**

When the small intestine is unable to produce enough lactase, the enzyme needed to break down lactose, the sugar found in milk, lactose intolerance develops (Lactose intolerance— symptoms and causes—Mayo Clinic). The nomenclature used to characterize lactose metabolism is quite ambiguous, which frequently leaves people perplexed **(Misselwitz B et al., 2019)**. When lactose is not absorbed and stays undigested in the gut, it can cause lactose malabsorption, which can result in bacterial fermentation in the intestinal lumen. After consuming lactose, this fermentation increases the osmotic load and causes intolerance symptoms **(Storhaug CL et al., 2017 , Forsgård RA et al.,2019).** Approximately 70% of adult humans worldwide have decreased lactase enzyme levels, with considerable regional and national variations **(Forsgård RA et al.,2019, EFSA Panel on Dietetic Products 2010, Bayless TM et al.,2017).** This condition results from the presence of other gastrointestinal illnesses or from lactase nonpersistence caused by genetics. Lactose malabsorption (LM) and lactose intolerance (LI) might arise from either of the two situations **(Swallow DM *et al.*,2003, Misselwitz B *et al.*,2013)**. People who have lactose intolerance (LI) are no longer recommended to abstain from all dairy products. Up to 5 grams of lactose per serving—roughly equal to 100 milliliters of milk—are tolerated by the majority of LI patients. When lactose is eaten with other nutrients, this tolerance level increases. It would be helpful in this regard to have a trustworthy resource for choosing items that don't exceed a person's lactose tolerance level **(Fassio F *et al.*, 2018).**

Non-Dairy Probiotics

Fruits and vegetables are recognized as wholesome foods and serve as an excellent medium for functional ingredients due to their abundance in beneficial nutrients such as phytochemicals, antioxidants, minerals, vitamins, and dietary fibers **(Slavin *et al.*,2012).** Their elevated nutrient and sugar content play a crucial role in facilitating probiotic growth, and when combined with their rapid passage through the acidic conditions of the stomach, they contribute to high probiotic cell viability (**Kandylis *et al.*, 2016).** Unlike dairy products, fruits and vegetables do not contain allergens, lactose, or cholesterol, making them more suitable for individuals with specific dietary concerns. Fruits, appreciated for

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their health benefits, refreshing nature, and appealing taste, have captured the interest of researchers in the development of probiotic beverages based on fruit juices **(Panghal *et al.*, 2018).**

Probiotic bacteria ferment the carbohydrates present in fruits, vegetables, grains, and legumes to produce probiotic food, which causes the emission of alcohol and gasses **(Panghal *et al.*, 2018).** Research has shown that the process of probiotifying fruit juice can result in tastes that are described as "bitter," "astringent," "dairy-like," "medicinal," "acidic," "salty," "dairy-like," "artificial," or "earthy" **(Luckow *et al.*, 2004)** The sensory qualities of the probiotic juice might vary depending on the kind of fruit, the probiotic organism, the temperature at which they are stored, and the addition of prebiotics and protectants **(Lebaka *et al.*, 2018).** Several investigations have demonstrated that probiotics have no effect on fruit juices' general acceptability.

Fruits and vegetables are considered to be very nutritious foods and provide an ideal foundation for functional components since they are rich in many beneficial elements, such as antioxidants, phytochemicals, minerals, vitamins, and dietary fibers **(Slavin *et al.*, 2012).** Probiotic development is aided by the high nutrient and sugar levels, and probiotic cell viability is preserved by their quick passage through the stomach's acidic environment **(Kandylis *et al.*, 2016).** Fruits and vegetables do not include allergies, lactose, or cholesterol, which can be harmful to some populations, unlike dairy products. Fruits provide a wholesome, energizing choice with a flavor and taste that appeals to people of all ages **(Panghal *et al.*, 2018).** Researchers looking into the creation of probiotic drinks based on fruit juice are interested in these qualities.

Fermented Beverages

One of the oldest techniques for digesting food is fermentation, which produces fermented products through the metabolic activities of a variety of microbiota. This microbiota is made up of both naturally occurring native microorganisms and purposefully introduced bacteria and yeasts, sometimes referred to as starting cultures. Foods are preserved by fermentation, which is made possible by the creation of organic acids. Additionally, this treatment improves their nutritional value and sensory qualities **(Rouhi, M *et al.*, 2013).** The kind of milk used, the specific probiotic microbe employed, and the addition of additional starter cultures are the main determinants of the important physicochemical properties of probiotic fermented milk products. Moreover, there is a range in the textures of fermented probiotic dairy products, ranging from semi-solid or thickened versions like drinking yogurt and villi to fluid drinks like acidophilus milk and kefir **(Jayawardana *et al.*, 2015).** The microbes used in starter cultures are very important to industry since they are essential to determining the texture and flavor of foods that have undergone fermentation. It's possible that these starting cultures don't naturally have probiotic properties. For example, because of their limited survivability in the digestive system, calling yogurt starter cultures such Streptococcus thermophilus and Lactobacillus delbrueckii spp. bulgaricus "probiotics" may not be accurate **(Senok *et al.*, 2005).** However, there are some positive health benefits linked to yogurt starter cultures, including enhanced immune system support and better lactose digestion **(Guarner *et al.*, 2005)**.

II. CONCLUSION

The trend towards functional foods—those containing bioactive ingredients like probiotics, fiber, and oligosaccharides—is on the rise. These foods not only support overall health but also help mitigate the risk of food-borne diseases. While dairy-based probiotic products have dominated the market, the growing demand for non-dairy alternatives has led to increased interest in fruit and vegetable-based probiotic beverages. These alternatives cater to those who are lactose intolerant, allergic to dairy, or prefer vegan and plant-based options.

Bitter gourd *(Momordicacharantia Linn.),* a plant known for its potential health benefits, has been explored in this study as a medium for probiotic fermentation. The objectives were to identify suitable probiotic strains, standardize fermentation protocols, and optimize conditions to enhance probiotic viability and activity in vegetable juice. Additionally, the study aimed to evaluate the physico-chemical, microbiological, and shelf-life parameters of the final product. The results showed that probiotic-enriched bitter gourd juice could be successfully developed with significant health benefits. The fermentation process increased the nutritional value of the juice, improved its flavor profile, and ensured the stability of probiotics during storage. The juice maintained a high count of viable probiotic cells, essential for delivering health benefits.

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Finally, the development of probiotic bitter gourd juice represents a promising advance in functional beverages. It offers a nutritious, non-dairy alternative with potential benefits for digestive health, appealing to health-conscious consumers and those with dietary restrictions. This study highlights the feasibility and advantages of incorporating probiotics into vegetable juices, paving the way for further innovations in the functional food and beverage industry.
The study supports the potential of probiotic bitter gourd juice as a functional beverage with health benefits and good consumer acceptability.

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