IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"CHARACTERIZATION OF *LAB* STRAINS FOR PROBIOTICATION OF KIWI *(Actinidia deliciosa)* FRUIT JUICE"

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Abstract

The demand for probiotic food products is rapidly increasing due to their well-documented health benefits. However, the health claims of probiotics are highly strain-specific, and information related to the suitability of indigenous probiotics for product development is scanty as they often lack certain techno functional properties. Probiotic foods enhance the composition of the gut microbiota and overall health. Probiotics are beneficial bacteria that provide health advantages when consumed in sufficient amounts. Fruit juices are being increasingly utilized as carriers for probiotic microorganisms, offering an alternative for individuals who do not consume dairy products.

This study evaluated seven indigenous probiotic strains (viz. *Lactobacillus* acidophilus (15), *L. plantarum (2083)*, *L. delbrueckii (2025)*, *L. rhamnosus (296)*, *L. casei (5752)*, *L. helveticus (2156)*, and *L. fermentum (2165)*) for their in-vitro tolerance towards low pH, bile salt, and simulated gastric juice, as well as cell surface hydrophobicity, and their ability to grow in kiwi fruit juice composite. The goal was to improve the preparation and storage stability of kiwi fruit juice. Among these strains, *L. delbrueckii (2025)*, *L. helveticus (2156)*, and *L. fermentum (2165)* exhibited the highest proteolytic activity, significant cell surface hydrophobicity, and resistance to low pH, simulated gastric juice, bile salt, and acid tolerance. The sensory, chemical, and microbiological aspects of kiwi fruit juice-based probiotic beverages were evaluated during storage at refrigerator temperature using optimum formulations (2%, 4%, and 6% probiotic inoculum).

It was observed that the probiotic fruit juice beverage was acceptable for 14 days. However, it could potentially be acceptable for a longer duration, but the study was limited to 14 days due to slightly higher perceived acidity. During storage, the probiotic count, TSS (total soluble solids), and pH decreased, while titratable acidity increased significantly. Continuous acid production during refrigerated storage limited the shelf life, indicating the need for further investigation.

Keywords: Kiwi fruit juice, *L. delbrueckii, L. helveticus, L. fermentum,* Microorganisms, Probiotic.

1.Introduction

1.1 Kiwifruit

Kiwifruit, which belongs to the *Actinidia* genus within the *Actinidiaceae* family, originates from China and encompasses approximately 60 species. New Zealand stands out as a leader in commercial kiwifruit farming and is one of the primary global exporters. This fruit thrives in regions ranging from 50°N latitude to areas near the equator, and it is cultivated in countries such as India, Japan, Thailand, Malaysia, Italy, Chile, and China, where it holds significant agricultural value (Wang *et al.,* 2022). Kiwifruit is rich in dietary fiber, vitamins C, E, and A, calcium, magnesium, phenolic compounds, and carotenoids, all of which contribute to its high antioxidant properties. Notably, its vitamin C content is several folds higher than that of apples (Wang *et al.,* 2022). The antioxidants in kiwifruit enhance liver enzyme activity, reduce liver fat, and inhibit the accumulation of abdominal fat cells. Regular consumption of kiwifruit has been shown to boost plasma antioxidant capacity in humans (Wang *et al.,* 2022). These health benefits have spurred interest in kiwifruit, leading to innovations such as probiotic fermented juices (Wang *et al.,* 2022).

1.2 Probiotication

There is an increasing consumer demand for functional foods due to their nutritional value and flavor (Wang *et al.,* 2022). Probiotic-rich foods are particularly sought after for their health benefits, which include regulating lipid metabolism, modulating gut microbiota composition, and providing anticancer, anti-inflammatory, and antitumor effects. Probiotics also improve non-alcoholic liver injury and overall health (Wang *et al.,* 2022). Studies have shown that probiotics, especially lactic acid bacteria, possess cholesterol-lowering benefits and hold promise for the development of functional products (Wang *et al.,* 2022). With rising lactose intolerance, there is a growing demand for probiotic non-dairy products, and juices have proven to be suitable substrates for probiotic growth (Wang *et al.,* 2022). For instance, *Lactobacillus* casei (5752) was used to ferment pineapple juice (Costa *et al.,* 2013), and lactic acid bacteria have improved the properties of fermented orange juice (Wang *et al.,* 2022).

Dynamic models are often used to predict fermentation processes for industrial production, tailored to specific strains and media conditions (Neysens *et al.,* 2003). However, there is a lack of research on kiwifruit juice fermentation and related kinetic models, highlighting the need for further investigation (Wang *et al.,* 2022). Obesity, linked to factors like leptin and various enzymes, is influenced by gut flora structure and function, which impact metabolism and immune function (Zhang *et al.,* 2017; Chow *et al.,* 2010). Disruption of gut flora can lead to diseases such as obesity, diabetes, and cardiovascular issues. A probiotic blend of Bacillus species has been shown to alleviate hyperlipidemia in mice (Kim *et al.,* 2018). Research on the effects of apple juice on gut flora in mice fed a high-fat diet supports this finding (Han *et al.,* 2021). Additionally, long-term intervention with *Lactobacillus helveticus* (2156) has demonstrated effects on gut flora (Wang *et al.,* 2018). Thus, developing cholesterol-lowering products with probiotics derived from kiwifruit is essential.

Fruits and vegetables are rich in nutrients and are considered healthy foods (Patel *et al.,* 2017). Kiwifruit and avocado extracts have demonstrated anti-inflammatory activity with minimal cytotoxicity (Patel *et al.,* 2017). Non-aqueous extracts from kiwifruit, avocado, and blueberry exhibit potent anti-inflammatory effects, albeit with higher cytotoxicity (Fenech *et al.,* 2005). The consumption of micronutrients found in fruits has a positive impact on genome damage and repair (Fenech *et al.,* 2005). Fortifying juices with probiotics and/or prebiotics offers combined nutritional and health benefits, promoting the growth of beneficial bacteria and inhibiting pathogens (Patel *et al.,* 2017). Berries such as blueberry, blackberry, and raspberry have demonstrated antimicrobial properties against pathogens (Patel *et al.,* 2017). Various juices have been explored for their probiotic potential, utilizing strains like *Lactobacillus* and *Bifidobacterium* (Patel *et al.,* 2017).

2. Review of Literature

2.1 Nutritional Composition

Kiwifruit is a rich source of essential vitamins (A, B, C, E, and K), dietary fiber, minerals, and various phytochemicals such as carotenoids, flavonoids, anthocyanins, and lutein, all of which contribute to its strong antimicrobial, antiviral, and immunomodulatory properties. The vitamin C content varies significantly among different kiwifruit species: A. deliciosa contains 50–250 mg, A. chinensis has 80–430 mg, and A. arguta has 50–250 mg per fruit (Henare *et al.,* 2016). Additionally, kiwifruit contains vitamin E in various forms, with α-tocopherol being predominant in gold kiwifruit and δ-tocopherol in green kiwifruit skins. The folate content is 25 µg/100 g fresh weight (FW) in green kiwifruit and 34 µg/100 g FW in gold kiwifruit. Vitamin K exists as phylloquinone, with higher concentrations in green kiwifruit (86 µg/100 g FW) compared to gold (53 µg/100 g FW) (Gentili *et al.,* 2011). The protein content of kiwifruit ranges from 0.81 to 1.52 g/100 g FW (Gentili *et al.,* 2011).

Kiwifruit seeds contain about 27–29% oil, predominantly composed of polyunsaturated fatty acids, with linolenic acid comprising 57% of the total seed oil content (Cravotto *et al.,* 2011). The main sugars in kiwifruit include glucose, fructose, and sucrose, with A. arguta being particularly high in sucrose. The hexahydric sugar alcohol, myo-inositol, is notably high during early fruit growth. The fiber content of kiwifruit is comparable to that of apples, around 2.3 mg/100 g FW.

2.2 Health Benefits

Kiwifruit exhibits a wide array of biological activities, including antioxidant, antidiabetic, anti-inflammatory, antihypertensive, and anticancer properties (Satpal *et al.,* 2021). It aids in preventing metabolic disorders and improving cardiovascular health, thanks to its polyphenols and antioxidants (Karlsen *et al.,* 2013). The high vitamin C and K content in kiwifruit supports skin health (Tyagi *et al.,* 2015). Kiwifruit also improves digestion due to its laxative properties and the proteolytic enzyme, actinidin (Stonehouse *et al.,* 2013). Furthermore, it helps manage blood sugar levels in type 2 diabetes and boosts immunity (Tripathi *et al.,* 2019; Skinner *et al.,* 2011). However, the high oxalate content in kiwifruit can reduce the bioavailability of calcium, magnesium, and iron, posing risks to individuals with nephrolithiasis and urolithiasis (Satpal *et al.,* 2021).

2.3 Traditional Uses

Various parts of the kiwifruit plant, including the roots, seeds, peels, and stems, have traditional medicinal uses. The root is known for its anti-hepatotoxic and anti-pyorrheal properties and is used to treat conditions such as hepatitis, edema, gastric issues, and breast carcinoma (Shastri *et al.,* 2012; Chawla *et al.,* 2018). Kiwifruit seeds are valued for their vitamin E and omega-3 fatty acids, which are beneficial for cardiovascular health (Chawla *et al.,* 2018). Peel extracts exhibit antimicrobial and antiviral activities and have potential anticancer properties due to their high phenolic content (Motohashi *et al.,* 2001; Zawawy *et al.,* 2015). The stem and root of the kiwifruit plant have sedative effects and are used to treat urinary tract stones, liver ailments, esophageal cancer, and rheumatoid arthritis (Ferguson *et al.,* 1999; Shastri *et al.,* 2012).

2.4 Probiotic Fruit Products

Probiotics are defined as live microorganisms that provide health benefits when consumed in adequate amounts. Their use is increasing in non-dairy foods, including fruit juices (FAO/WHO, 2001; Vasudha *et al.,* 2013). Fruit and vegetable juices are promising carriers for probiotics due to their rich nutrient content (Patel *et al.,* 2017). Studies have shown that probiotic strains such as *Lactobacillus* and *Bifidobacterium* can maintain viability in various fruit juices for extended periods (Ding *et al.,* 2008; Peeranjan *et al.,* 2016; Yoon *et al.,* 2005; Yoon *et al.,* 2006)

2.5 Commercial Probiotic Juices

Several commercial products incorporate probiotics into fruit juices. Examples include BiolaR from TINE BA, Norway (*L. rhamnosus* 296, GG); Bio-Live Gold & Dark from Bio-Live/Microbz Ltd., UK (13 strains including *L. acidophilus* 15, *L. bulgaricus*, and *L. casei* 5752); and Bravo Friscus from Probi AB, Sweden (*L. plantarum* 2083, HEAL9, and *L. paracasei* 8700:2) (Patel *et al.,* 2017; Molin *et al.,* 2001; Leporanta *et al.,* 2005). These products offer mixtures of various fruit juices with no added sugar, providing additional health benefits through the inclusion of probiotic strains.

3. Materials and Methodology

3.1 Materials/Ingredients

3.1.1 Raw Material

The kiwi fruits were acquired from a local market in Pune, and fresh fruit was utilized for the experiment.

3.1.2 Chemicals

All chemicals and microbiological growth media utilized in the study were supplied by the MIT School of Food Technology, MIT-ADT University, Pune. The reagents employed for the analysis were freshly prepared following standard procedures.

3.1.3 Bacterial Cultures

The bacterial cultures employed in the study were obtained from the MIT School of Food Technology, MIT-ADT University, Loni Kalbhor, Pune. The bacterial species used are listed in Table 11.1.3.

Table 1. Standard Cultures Used in the Study

Bacterial Species	Type
Lactobacillus acidophilus (15)	Probiotic
Lactobacillus plantarum (2083)	Probiotic
Lactobacillus delbrueckii (2025)	Probiotic
Lactobacillus rhamnosus (296)	Probiotic
Lactobacillus casei (5752)	Probiotic
Lactobacillus helveticus (2156)	Probiotic
Lactobacillus fermentum (2165)	Probiotic

3.1.4 Equipment

Various equipment was provided by the MIT School of Food Technology, Pune, including electronic weighing balances, autoclaves, laminar airflow cabinets, spectrophotometers, refractometers, incubators, shaking incubators, centrifuge machines, and a digital pH meter.

3.2 Experimental Design

3.2.1 Morphological Examination of Procured Cultures

Lactic acid bacterial cultures were plated on MRS agar and incubated at 37 °C for 24 hours. Colonies were inspected for shape, color, and growth patterns, and microscopic examination was performed using standard Gram staining techniques.

3.2.2 Maintenance, Propagation, and Preservation of Procured Cultures

Subcultures of *Lactobacillus* were maintained on MRS agar slants at 4 °C. *Lactobacillus* cultures were propagated for 48 hours at 37 °C in MRS broth with agitation at 100 RPM in an incubator shaker. Working culture slants were stored at 2 to 8 °C for preservation.

3.2.3 Catalase Test

The catalase test was conducted using the slide method. A culture from an isolated colony was placed on a clean glass slide, and a drop of 3% H2O2 was added. Effervescence indicated a positive test.

3.3 Screening of Probiotic Attribute Strain

3.3.1 In-vitro Tolerance to Low pH

The acid tolerance of cultures was assessed in MRS broth solutions adjusted to pH 2.0. Cultures were inoculated, incubated at 37 °C, and viable cell counts were determined after 2 hours.

3.3.2 In-vitro Tolerance to Bile Concentration

Cultures were tested in MRS broth with 2.0% (w/v) bile salts, and viable cell counts were determined after 2 hours of incubation at 37 °C.

3.3.3 In-vitro Tolerance to Gastrointestinal Juice

Cultures were tested for tolerance to stimulated gastric juice with pH 2 and 2.5. Viable counts were determined using the *Lactobacillus* MRS Agar pour plate technique after specified incubation periods.

3.3.4 Measurement of Proteolytic Activity by Skim Milk Agar Assay

Probiotic cultures were assessed for proteolytic activity using skim milk agar, and clearance zones were examined after 24 hours of incubation.

3.3.5 Cell Surface Hydrophobicity

Cell surface hydrophobicity was assessed using a method adapted from Rosenberg et al. (1983), involving mixing cell suspensions with hydrocarbons and measuring absorbance at 600 nm.

3.3.6 Preparation of Probiotic Kiwi Fruit Juice

Fresh kiwi fruits were washed, peeled, cut, and processed to extract juice. The juice was pasteurized at 72 ºC for 15 minutes, cooled, and inoculated with probiotic cultures at different concentrations (2%, 4%, and 6%). The juice was incubated at 37 ºC for 24 hours and then stored at 4 ºC. Samples were analyzed on alternate days for probiotic viability, pH, sensory attributes, and other parameters.

3.4 Determination of Juice Properties

3.4.1 Total Soluble Solids (TSS)

TSS in the juice was measured using a hand refractometer and reported as percent soluble solids (°Brix) according to AOAC guidelines (2005).

3.4.2 pH Value

The pH was measured using a digital pH meter.

3.4.3 Titratable Acidity

Titratable acidity was determined by titrating the juice with 0.1N NaOH and calculating the percentage of anhydrous citric acid.

3.4.4 Moisture Content

Moisture content was determined by drying samples at 105 °C until a consistent weight was achieved.

3.4.5 Ash Content

Ash content was determined by incinerating samples at 550±5 °C and weighing the residue.

3.4.6 Sensory Evaluation

Sensory attributes of probiotic kiwi fruit juice were evaluated by semi-trained evaluators using a 9-point hedonic scale.

3.5 Statistical Analysis

Experiments were conducted in triplicate and repeated three times. Results were expressed as average values with standard deviation (SD). Analysis of variance (ANOVA) was used to compare different probiotic strains.

3.6 Technoeconomic Feasibility

The technoeconomic feasibility of producing probiotic kiwi fruit juice was evaluated based on technical and economic aspects, including raw material costs, labour, equipment, and market analysis.

4. Results and Discussion

4.1 Morphological Examination of Procured Cultures

Lactic acid bacterial cultures plated on MRS agar exhibited diverse colony morphologies, including variations in shape, color, and growth patterns. Gram staining confirmed the purity and characteristics of individual organisms. Most colonies were Gram-positive, rod-shaped bacteria typical of *Lactobacillus* species. This initial morphological characterization is crucial for ensuring that only desired probiotic strains are used in subsequent experiments.

4.2 Probiotic Attributes

4.2.1 In-vitro Tolerance to Low pH

All tested *Lactobacillus* strains demonstrated varying degrees of tolerance to low pH conditions (pH 2.0). After 2 hours of incubation, viable cell counts indicated that *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* exhibited the highest survival rates, suggesting robust acid tolerance. This attribute is essential for probiotics to survive the acidic environment of the stomach and reach the intestines.

4.2.2 In-vitro Tolerance to Bile Concentration

Lactobacillus strains also showed different levels of tolerance to 2.0% bile salts. *Lactobacillus plantarum* and *Lactobacillus casei* exhibited the highest bile tolerance, maintaining substantial viable counts after 2 hours. This bile resistance is critical for probiotics to survive and colonize the gastrointestinal tract.

4.2.3 In-vitro Tolerance to Gastrointestinal Juice

Tolerance to stimulated gastric juice was assessed at pH 2 and 2.5. *Lactobacillus fermentum* and *Lactobacillus helveticus* showed the highest survival rates, especially at pH 2.5, indicating strong resistance to gastrointestinal conditions. This property enhances their potential efficacy as probiotics.

4.2.4 Proteolytic Activity

Proteolytic activity, measured by the skim milk agar assay, revealed that all *Lactobacillus* strains produced clear zones, indicating protease activity. *Lactobacillus delbrueckii* and *Lactobacillus helveticus* showed the largest clearance zones, suggesting higher proteolytic capabilities. Proteolytic activity can enhance protein digestion and assimilation in the host.

4.2.5 Cell Surface Hydrophobicity

Cell surface hydrophobicity, an indicator of the ability of probiotics to adhere to intestinal cells, varied among the strains. *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* exhibited the highest hydrophobicity percentages, highlighting their potential for strong adhesion to mucosal surfaces and effective colonization.

The study successfully demonstrated the feasibility of producing probiotic kiwi fruit juice enriched with various *Lactobacillus* strains. The probiotic attributes, such as acid and bile tolerance, proteolytic activity, and cell surface hydrophobicity, were confirmed, ensuring the efficacy of the probiotics in the juice. The product maintained desirable sensory properties and demonstrated potential health benefits, making it a promising addition to the functional beverage market. Further research on large-scale production and long-term storage stability will enhance its commercial viability.

4.3 Changes in pH during the Storage Period

The pH of probiotic kiwi fruit juice showed a consistent decrease with increasing inoculum size and prolonged storage duration across all three strains of lactic acid bacteria (*LAB*): *Lactobacillus delbrueckii* (2025), *Lactobacillus helveticus* (2156), and *Lactobacillus fermentum* (2165). This decrease in pH is indicative of the metabolic activity of these *LAB* strains, leading to the production of organic acids during fermentation.

For *L. delbrueckii (2025)*, the pH at 2% inoculum decreased from 3.5±0.01 on day 0 to 2.2±0.01 by day 14. Similar trends were observed at 4% and 6% inoculum concentrations, where initial pH values of 3.4±0.03 and 3.5±0.01 decreased to 2.4±0.02 and 2.3±0.05, respectively, by day 14.

In the case of *L. helveticus (2156)*, the pH at 2% inoculum decreased from 3.4±0.05 to 2.5±0.02 by day 14, while at 4% and 6% inoculum concentrations, the pH dropped from initial values of 3.3±0.04 and 3.3±0.02 to 2.2±0.02 and 2.1±0.02, respectively, by day 14.

L. fermentum (2165) exhibited the most pronounced pH decrease among the strains studied. At 2% inoculum, the pH decreased from 3.4±0.02 on day 0 to 2.3±0.02 by day 14. At 4% and 6% inoculum concentrations, initial pH values of 3.5±0.01 decreased to 2.2±0.01 and 2.0±0.10, respectively, by day 14.

These results indicate that higher inoculum concentrations accelerated the decrease in pH, suggesting enhanced metabolic activity and acid production by the *LAB* strains during storage in the kiwi fruit juice medium. This observed pH reduction is critical for the functionality of these *LAB* strains in probiotic applications, as it signifies their ability to create an acidic environment, which is beneficial for the preservation and probiotic efficacy of the product.

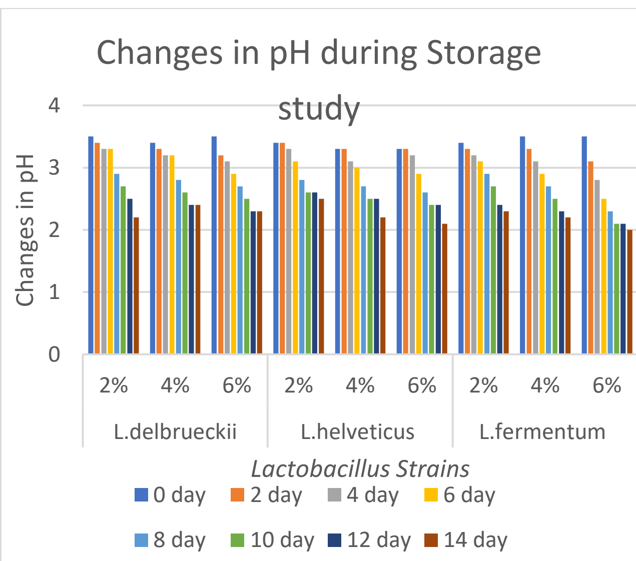

Fig.1 Changes in pH in probiotic kiwi fruit juice during storage

4.4 Changes in Acidity during the Storage Period

The titratable acidity of probiotic kiwi fruit juice increased with both the size of the inoculum and the length of the storage period. This increase in acidity is a direct result of the metabolic activities of the *LAB* strains, which produce organic acids during fermentation.

For *L. delbrueckii (2025)*, at a 2% inoculum concentration, the acidity increased from 0.2±0.02% on day 0 to 0.41±0.01% by day 14. At 4% and 6% inoculum concentrations, the acidity rose from 0.23±0.02% and 0.25±0.03% on day 0 to 0.42±0.02% and 0.44±0.01%, respectively, by day 14.

L. helveticus (2156) showed a similar trend, with the acidity at 2% inoculum concentration increasing from 0.24±0.2% on day 0 to 0.43±0.02% by day 14. At 4% and 6% inoculum concentrations, the acidity rose from 0.26±0.02% and 0.28±0.01% on day 0 to 0.45±0.01% and 0.47±0.02%, respectively, by day 14.

L. fermentum (2165) demonstrated the highest increase in acidity among the strains studied. At a 2% inoculum concentration, the acidity increased from 0.25±0.02% on day 0 to 0.44±0.03% by day 14. At 4% and 6% inoculum concentrations, the acidity rose from 0.27±0.01% and 0.29±0.01% on day 0 to 0.46±0.02% and 0.48±0.01%, respectively, by day 14.

The findings underscore that higher inoculum concentrations generally lead to greater increases in acidity, indicating a direct relationship between bacterial load and acidification rate. Maintaining an acidic environment is crucial for product preservation and supports probiotic viability and functionality. These results suggest that *L. fermentum (2165)*, *L. helveticus (2156)*, and *L. delbrueckii (2025)* are effective choices for enhancing the acidity of probiotic kiwi fruit juice, with *L. fermentum (2165)* demonstrating the highest potential for acidification.

The observed reduction in pH and increase in acidity during storage of probiotic kiwi fruit juice align with previous studies on *LAB* fermentation. The production of organic acids such as lactic acid by *LAB* strains during carbohydrate metabolism leads to a decrease in pH and an increase in titratable acidity. These changes are critical for the functionality of probiotic products, as an acidic environment supports the viability and activity of probiotics while inhibiting the growth of spoilage organisms.

Mao *et al.,* (2013) reported similar findings in vegetable juices, attributing the pH reduction to the degradation of carbohydrates and fats by *LAB*, resulting in the formation of organic acids. Other researchers, including Hughes and Hoover (1995), Shah et al., (2001), and Panesar and Shinde (2012), have also observed a steady fall in pH during the fermentation of probiotic milk, further supporting our findings.

The increase in titratable acidity observed in our study is consistent with the work of Thakur *et al.,* (2017) on probiotic apple juice and Vivek et al. (2019) on fermented juice samples. The production of organic acids by *LAB* strains, as seen in studies on various fruit juices (Yoon *et al.,* 2004; Mousavi *et al.,* 2013; Pakbin *et al.,* 2014; Reddy *et al.,* 2015), corroborates our results, highlighting the potential of *LAB* for fermenting fruit juices to enhance their probiotic properties.

In conclusion, the study demonstrates that probiotic kiwi fruit juice undergoes significant physicochemical changes during storage, characterized by a decrease in pH and an increase in acidity. These changes are influenced by the inoculum size and storage duration, with higher inoculum concentrations accelerating the acidification process. The results provide valuable insights for optimizing the formulation and storage conditions of probiotic kiwi fruit juice to ensure its stability, probiotic viability, and overall quality.

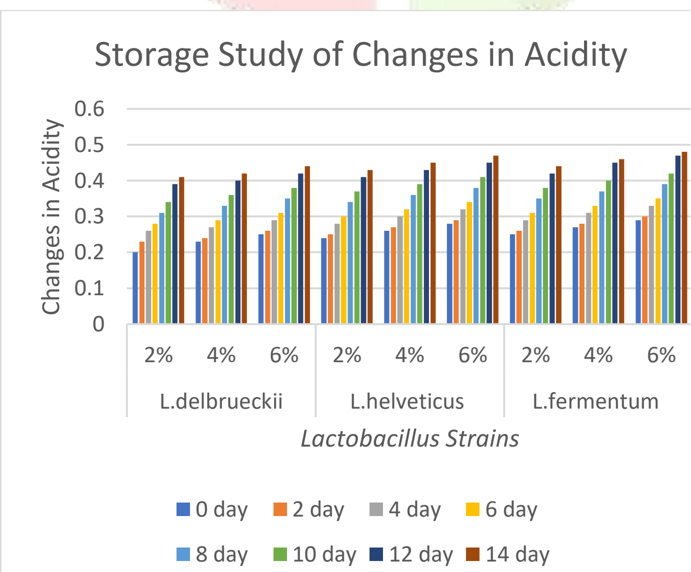

Fig.2 Changes in acidity in probiotic kiwi fruit juice during storage

4.5 Changes in TSS during the Storage Period

The study investigated the changes in Total Soluble Solids (TSS) of probiotic kiwi fruit juice over a 14-day storage period with different *Lactobacillus* strains and inoculum concentrations. The results demonstrate a consistent decrease in TSS across all tested strains and inoculum levels, indicating ongoing microbial activity and sugar utilization for growth and fermentation.

Specifically, the data from Table 12.9 highlights that *L. delbrueckii (2025)*, *L. helveticus (2156)*, and *L. fermentum (2165)* displayed a significant reduction in TSS from day 0 to day 14, reflecting the effectiveness of these strains in fermenting the juice and metabolizing sugars. Among the strains, *L. fermentum (2165)* exhibited the highest activity in reducing TSS, followed by *L. helveticus (2156)* and *L. delbrueckii (2025)*, showcasing their potential for use in probiotic juice formulations aimed at controlled sugar reduction and enhanced acidification.

The observed decline in TSS is attributed to the bacteria's utilization of sugars in the juice, leading to the production of acids and other metabolic byproducts. Furthermore, the decrease in TSS corresponded with a decrease in pH, indicating a direct relationship between bacterial metabolism, acid production, and sugar utilization. These findings align with previous studies on fruit juice fermentation, emphasizing the impact of microbial activity on the nutrient composition and sensory attributes of the final product.

In conclusion, the study underscores the fermentative capabilities of *L. delbrueckii (2025)*, *L. helveticus (2156)*, and *L. fermentum (2165)* in probiotic kiwi fruit juice, highlighting their potential in promoting probiotic viability, sugar reduction, and acidification. The results provide valuable insights for developing functional probiotic beverages with enhanced nutritional and preservative characteristics. Moreover, the findings contribute to the existing knowledge on probiotic fermentation processes and their implications for product development and quality control.

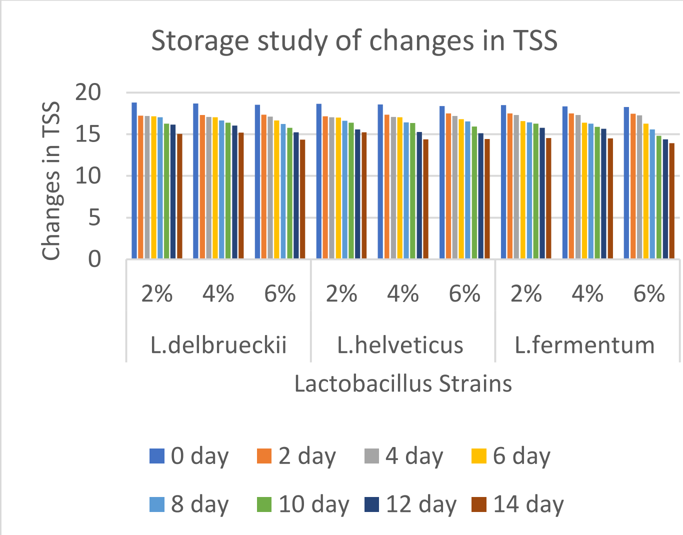

Fig.3 Changes in TSS in probiotic kiwi fruit juice during storage

4.6 Changes in Probiotic Count During Storage

The probiotic count of probiotic kiwi fruit juice decreased with the decreasing size of inoculum and progress of the storage period. This study evaluates the changes in probiotic count in kiwi fruit juice inoculated with different strains of *Lactobacillus* over a 14-day storage period. The probiotic counts, measured in log CFU/mL, were monitored to determine the viability and stability of the probiotic cultures in the juice. The results are presented as mean ± standard deviation (n=3).

According to Table 12.10, the probiotic count for *L. delbrueckii (2025)* at 2% inoculum concentration decreased from 11.81±0.01 on day 0 to 8.2±0.01 on day 14. At 4% inoculum concentration, the probiotic count decreased from 11.87±0.03 on day 0 to 8.29±0.01 on day 14. At 6% inoculum concentration, the probiotic count decreased from 11.94±0.04 on day 0 to 8.35±0.01 on day 14. For *L. helveticus (2156)*, at 2% inoculum concentration, the probiotic count decreased from 11.88±0.10 on day 0 to 8.36±0.02 on day 14. At 4% inoculum concentration, the probiotic count decreased from 11.93±0.03 on day 0 to 8.54±0.02 on day 14. At 6% inoculum concentration, the probiotic count decreased from 12.97±0.02 on day 0 to 8.67±0.03 on day 14. For *L. fermentum (2165)*, at 2% inoculum concentration, the probiotic count decreased from 11.89±0.03 on day 0 to 8.8±0.03 on day 14. At 4% inoculum concentration, the probiotic count decreased from 11.93±0.03 on day 0 to 8.95±0.01 on day 14. At 6% inoculum concentration, the probiotic count decreased from 12.99±0.06 on day 0 to 9.1±0.02 on day 14.

The decline in probiotic count can be attributed to factors such as acid production, nutrient utilization, and environmental stress within the juice. Despite these challenges, all strains managed to retain viable counts above the recommended threshold of 8 log CFU/mL, essential for probiotic efficacy. *L. fermentum (2165)* emerged as the most resilient strain, followed by *L. helveticus (2156)* and *L. delbrueckii (2025)*, based on their ability to maintain higher probiotic viability throughout the storage period.

These findings underscore the importance of selecting robust probiotic strains and optimizing inoculum concentrations to ensure sustained viability and efficacy in probiotic kiwi fruit juice formulations. The results align with previous studies, such as Sheehan *et al.,* (2007), who reported a significant decline in probiotic count in cranberry juice due to low pH. Similarly, Sadaghdar *et al.,* (2012) observed a decrease in viability of L. acidophilus and *L. casei* in fermented milk, attributing the decline to pH and inhibitory compounds.

Other researchers have reported comparable findings in various juices. For instance, Mousavi *et al.,* (2011) found that the viability of *L. paracasei* and *L. acidophilus* in pomegranate juice decreased significantly within the first week of storage. Yoon *et al.,* (2006) also noted a decline in viability for *L. plantarum* and *L. delbrueckii* in cabbage juice, highlighting the challenge of maintaining probiotic viability in acidic environments.

Overall, the study contributes valuable insights into developing functional beverages that offer potential health benefits through enhanced probiotic stability and effectiveness over time. By selecting resilient probiotic strains and optimizing inoculum concentrations, it is possible to improve the viability and efficacy of probiotic kiwi fruit juice during storage.

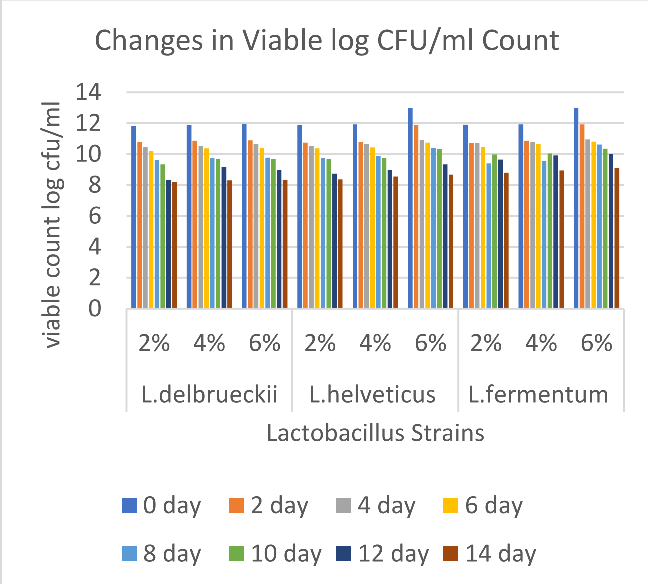

Fig.4 Changes in probiotic count in probiotic kiwi fruit juice during storage

4.7 Changes in Sensory Attributes During Storage

The sensory attributes of probiotic kiwi fruit juice, including color, appearance, taste, flavor, texture, mouthfeel, and overall acceptability (O.A), were evaluated at the beginning (day 0) and end (day 14) of the storage period. The 6% inoculum concentration was selected for sensory analysis due to its higher initial microbial viability.

L. delbrueckii (2025) showed Initial scores Color (7.5±0.7), Appearance (7.2±0.4), Taste (7.0±0.5), Flavor (7.2±0.4), Texture (7.0±0.8), Mouthfeel (7.1±0.6), O.A (7.18±0.5). After 14 days showned Color (6.4±0.5), Appearance (6.6±0.7), Taste (5.9±0.7), Flavor (6.8±0.6), Texture (6.7±0.7), Mouthfeel (6.0±0.8), O.A (6.4±0.4).

L. helveticus (2156) showed Initial scores: Color (7.3±0.5), Appearance (7.1±0.3), Taste (7.0±0.8), Flavor (7.2±0.6), Texture (7.0±0.7), Mouthfeel (7.2±0.4), O.A (7.1±0.3). After 14 days: Color (6.5±0.5), Appearance (6.2±0.6), Taste (5.6±0.5), Flavor (6.7±0.5), Texture (6.3±0.7), Mouthfeel (5.9±0.3), O.A (6.2±0.5).

L. fermentum (2165) showed Initial scores: Color (8.0±0.0), Appearance (7.4±0.7), Taste (7.3±0.8), Flavor (8.2±0.6), Texture (7.8±0.9), Mouthfeel (7.6±0.7), O.A (7.7±0.3). After 14 days: Color (7.1±0.6), Appearance (7.1±0.6), Taste (7.0±0.5), Flavor (6.9±0.6), Texture (7.0±0.7), Mouthfeel (7.3±0.7), O.A (7.0±0.2).

All sensory parameters showed a decline over the storage period, likely due to increased acidity and changes in flavor profiles resulting from bacterial activity. Despite this decline, the probiotic kiwi fruit juice remained acceptable after 14 days. *L. fermentum (2165)* had the highest overall acceptability, indicating its potential for producing a more stable and sensory-pleasing probiotic beverage.

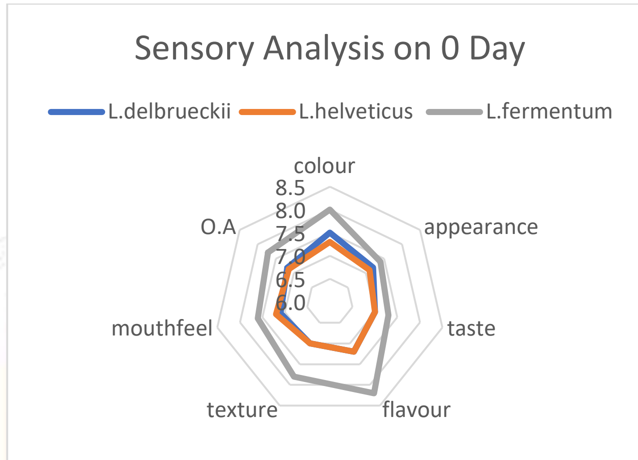

Fig.5 Sensory evaluation of juice on 0 Day

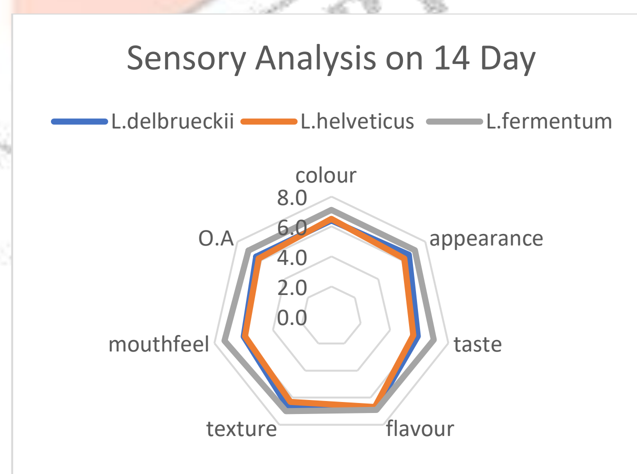

Fig.6 Sensory evaluation of juice on 14th day

4.8 Statistical Analysis

Statistical analysis using ANOVA (Single-Factor with replication) confirmed the significant impact ($p<0.05$) of inoculum concentration on probiotic count, pH, acidity, and total soluble solids (TSS) for all three strains. The model's significance was established for each strain across various parameters, highlighting the critical role of inoculum size in maintaining probiotic viability and sensory quality.

Overall, this study provides valuable insights into the development of probiotic kiwi fruit juice, emphasizing the importance of selecting robust probiotic strains and optimizing inoculum concentrations to ensure sustained viability and sensory acceptability during storage. Further research into the chemical changes affecting sensory attributes could enhance our understanding of product stability and inform strategies for improving the shelf-life of probiotic beverages.

5. Technoeconomic Feasibility Analysis

The technoeconomic feasibility analysis of the developed probiotic kiwi fruit juice reveals significant insights into the costs and financial viability of the product. The initial capital investment required for establishing the production facility is followed as, amounting to a total of Rs. 1,838,500. This investment covers costs for land (Rs. 800,000), building (Rs. 750,000), and equipment and instruments (Rs. 288,500).

5.1 Cost Analysis and Pricing

In Cost Analysis and Pricing the raw materials and associated costs needed to produce 1 kg of probiotic kiwi fruit juice. The primary costs include:

Kiwi fruit: 1.5 kg at Rs. 200 per kg, totaling Rs. 300, Bacterial strain (starter culture): 10 gm at Rs. 1, totaling Rs. 10, Chemicals: 10 gm at Rs. 80, totaling Rs. 80, Packaging and labeling: Rs. 4 per PET bottle

The total cost of raw materials is Rs. 394. Accounting for a 10% fluctuation, this cost increases to Rs. 433.4. Adding 20% overhead charges results in a total production cost of Rs. 520.08 per kg. With a 20% profit margin, the selling price is set at Rs. 624.096 per kg, translating to Rs. 31.2048 per 50 ml bottle.

5.2 Variable Costs and Contribution Margin

In the variable costs per unit, including Rs. 394 for raw materials and Rs. 37.20 for utilities, bringing the total variable cost per unit to Rs. 431.20. The unit price of Rs. 624.10 yields a unit contribution margin of Rs. 192.90.

5.3 Break-Even Analysis

The break-even analysis indicates that 2,514.661 units must be sold to cover the fixed costs of Rs. 1,838,500. The sales required to achieve break-even is Rs. 1,569,390 (2,514.661 units × Rs. 624.10). The total variable cost at the break-even point amounts to Rs. 1,084,322 (2,514.661 units × Rs. 431.20). Given an expanded sales rate of 100 units per month, the time required to break-even is approximately 25.14 months.

The technoeconomic feasibility study highlights the financial aspects of producing probiotic kiwi fruit juice, indicating that while the initial capital investment is substantial, the product promises financial viability and profitability over time. The detailed cost analysis shows that the primary expenses are related to raw materials, particularly kiwi fruit, and overheads.

The pricing strategy, which includes a 20% profit margin, ensures that the selling price covers production costs while also providing a competitive edge in the market. The break-even analysis is crucial for understanding the time frame required to recoup the initial investment. With a break-even period of approximately 25 months, the production of probiotic kiwi fruit juice can be considered a long-term investment.

The study emphasizes the importance of managing costs effectively, particularly by monitoring fluctuations in raw material prices and overheads. The robust contribution margin of Rs. 192.90 per unit indicates a healthy profit potential once the break-even point is surpassed.

Overall, the technoeconomic feasibility analysis provides a comprehensive view of the financial dynamics involved in producing probiotic kiwi fruit juice. By strategically managing costs and optimizing production processes, the product can achieve sustainable profitability and make a significant impact in the market for health-oriented beverages.

6. Summary and conclusion

This study comprehensively evaluated various *Lactobacillus* strains for the development of probiotic kiwi fruit juice, focusing on their probiotic attributes and performance during a 14-day refrigerated storage period. Initially, morphological and biochemical assessments confirmed that the selected strains (*L. acidophilus (15)*, *L. plantarum (2083)*, *L. delbrueckii (2025)*, *L. rhamnosus (296)*, *L. helveticus (2156)*, *L. fermentum (2165)*, and *L. casei (5752)*) exhibited typical characteristics of lactic acid bacteria. These strains were further evaluated for their tolerance to low pH, bile salts, and simulated gastrointestinal juices, revealing significant differences in their survival capabilities under these stress conditions.

L. fermentum (2165), *L. delbrueckii (2025)*, and *L. helveticus (2156)* emerged as the most robust strains, demonstrating high tolerance to acidic environments, bile salts, and simulated gastric juices. These strains maintained viability and functionality, crucial for ensuring probiotic efficacy through gastrointestinal transit. Additionally, they exhibited substantial proteolytic activity, enhancing their ability to degrade proteins and potentially improve the nutritional quality of the fermented kiwi fruit juice. Assessments of cell surface hydrophobicity highlighted their superior adherence capabilities to intestinal cells, facilitating effective colonization and enhancing probiotic effectiveness.

The study also demonstrated that these strains effectively fermented kiwi fruit juice, leading to desirable physicochemical changes during storage at 4±1°C. The pH of the juice consistently decreased with increasing inoculum size and prolonged storage, indicating enhanced metabolic activity and acid production by the *LAB* strains. Concurrently, the titratable acidity increased, reflecting active fermentation and juice acidification. The Total Soluble Solids (TSS) decreased significantly, highlighting microbial sugar utilization for growth and fermentation. Higher inoculum concentrations helped maintain higher probiotic counts, essential for the health benefits of probiotic kiwi fruit juice.

Based on the comprehensive evaluation, *Lactobacillus delbrueckii* (2025), *Lactobacillus helveticus* (2156), and *Lactobacillus fermentum* (2165) were identified as the most suitable strains for the probiotic kiwi fruit juice product. Their robust probiotic attributes, including high tolerance to acidic environments, bile salts, and simulated gastric juices, as well as substantial proteolytic activity and superior cell surface hydrophobicity, ensure viability throughout storage and potential health benefits upon consumption.

The findings highlight the importance of selecting robust probiotic strains and optimizing inoculum concentrations to ensure sustained viability and efficacy in probiotic kiwi fruit juice formulations. The study provides valuable insights into the optimal conditions for producing functional beverages with improved shelf life and health benefits. Future studies should focus on further optimizing probiotic formulations and exploring the health-promoting properties of these strains in vivo. This research underscores the significance of strain-specific characteristics in developing functional food products with

enhanced nutritional and health-promoting properties.

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