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Process optimization of mango probiotic beverage

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Abstract

The present study used a well-known probiotic strain called *Lactobacillus plantarum* NCDC 19 to prepare a mango-based probiotic drink. The fermentation process took place for 24 h at 37°C under microaerophilic conditions. During this time, the juice was evaluated for both physicochemical and phytochemical characteristics. The researchers also evaluated the survival rate of the probiotic strain after it was kept at a temperature of 10°C for a period of four weeks. The quantity of living probiotic cells grew steadily throughout the fermentation process until it peaked at 9.55 log colony forming units (CFU) per milliliter. As fermentation continued, the pH of the juice decreased, while the titratable acidity and antioxidant activity both increased. It was found that *L. plantarum* was particularly effective at consuming sugar and lowering the pH of the mango pulp. The research findings suggest that mango pulp is a suitable ingredient that can be used in the preparation of probiotic beverages.

1. Introduction

Numerous studies have investigated the relationship between fruit consumption and its potential health benefits. The food industry places a high priority on the research and development of products that promote health, as emphasized by Min *et al.* (2019). One promising area of research is the use of live probiotic bacteria, which have been associated with several health benefits such as reducing serum cholesterol levels, preventing intestinal infections, improving digestive function, strengthening the immune system, lowering the risk of colon cancer, improving lactose digestion, and reducing blood ammonia levels, as reported by Pradhan *et al.* (2020).

Mango is considered the most important fruit in pomology and is widely cultivated, occupying approximately one-third of the total area dedicated to fruit production, as noted by Saadat and Gupta (2018). The sweetened pulp of mango contains 26.0% carbohydrates (including added sugars), 1.0% protein, and 4 mg of sodium per 100 g of pulp. To standardize the pulp, sugar syrup and citric acid are typically added to achieve a 14-18° brix sweetness level and 0.65-0.75% acidity (as citric acid), respectively. Bintsis *et al.* (2018) reported that *Lactobacillus* is the most extensive genus of lactic acid bacteria and is commonly used in producing fermented dairy products, sourdough, meat, and vegetable foods. Moreover, this genus has potential applications as a probiotic.This study aimed to evaluate if mango pulp can be used as a suitable substrate for producing probiotics. The study also looked at the growth rate, how the lactic acid bacteria strains metabolize the substrate and analyzed the ability of the bacteria to survive under cold storage conditions.

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2. Materials and Methods

This study was carried out in the laboratory of the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The raw materials were procured from the local market of Varanasi. The process flow chart of this study is shown in Figure 1. Various materials used and methods followed during the period of investigation relating to technological, and analytical aspects are described in sequence.

2.1 Culture activation and mango pulp preparation

The probiotic culture NCDC 19 was procured from the National Collection of Dairy Cultures (NCDC), NDRI, Karnal, Haryana, India. The microorganisms were cultivated in MRS Broth at a temperature of 37ºC for 24 h. Then, they were subjected to activation in skim milk twice and used as an inoculum as per Chand *et al.* (2021). Alphonso mango pulp was purchased from Nature's First India Private Limited and its total solid (TS) content was checked. The total solid content of the mango pulp was adjusted to 10º brix for the preparation of the mango probiotic drink. 100 ml sterile flasks each containing 60 ml of pasteurized mango juice were taken and probiotic culture of *L. plantarum* NCDC 19 activated in skim milk was inoculated at a 1% rate and the sample was incubated at 37ºC for 24 h.

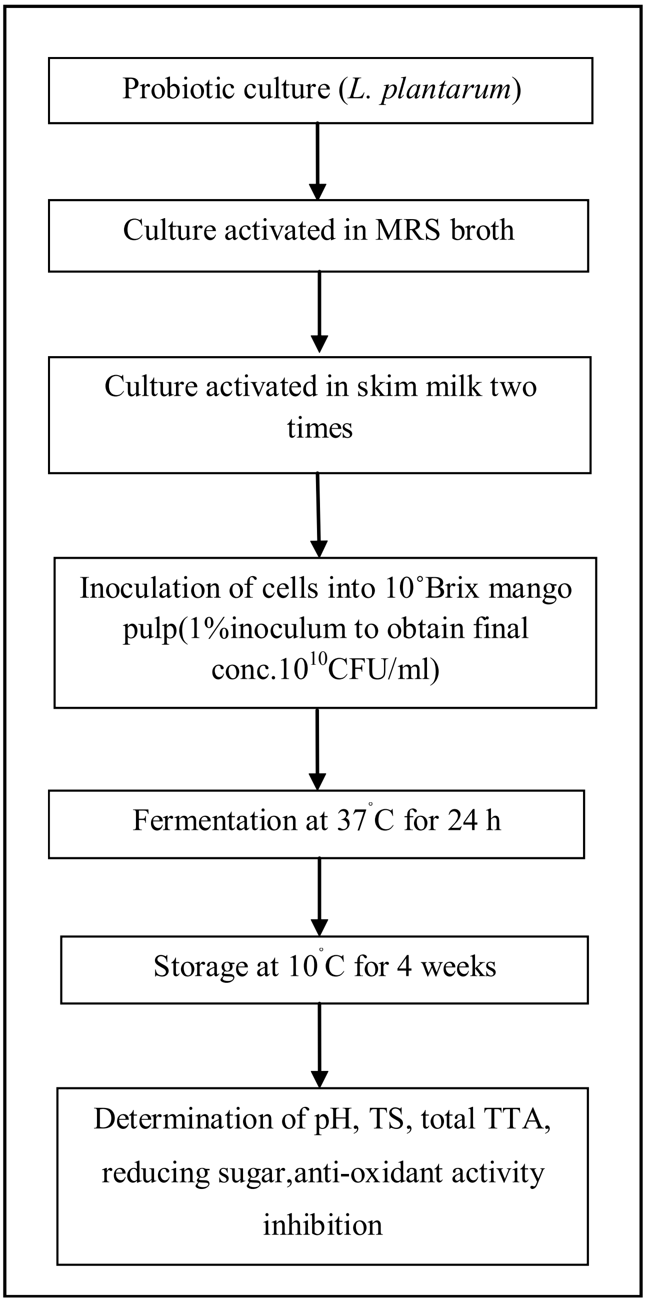

Figure 1: Flow diagram of the optimization process of mango probiotic beverage.

2.2 Microorganisms and fermentation

To determine the viable cell count of the fermented mango pulp, the researchers used the standard plate count method with an MRS medium. Samples were collected from the fermented mango pulp at different time intervals (0, 4, 8, 12, and 24 h), and 1 ml was taken from each sample. The samples were then diluted using a 10-fold dilution series.The diluted samples were then spread on MRS agar plates, which were allowed to solidify for half an hour and then placed in an incubator set to 37ºC for 24 to 48 h to determine the viable cells. After incubation, plates were withdrawn from the incubator and typical colonies of *Lactobacillus* (white, small, circular, convex) were counted manually (Shakibaie *et al.,* 2017). The MRS plates were placed in an incubator at a temperature of 37ºC for a duration of 24 to 48 h. After fermentation, the colonies appeared small, white and convex-shaped. These colonies were then counted, and the result was expressed in terms of colony-forming units per milliliter (CFU/ml). To evaluate the effect of cold storage on the probiotic culture viability, the fermented samples were stored at a temperature of 10ºC for four weeks, and the probiotic bacteria's survival rate was monitored over this duration. The number of microorganisms was measured every week and reported as CFU/ml.

2.3 Physicochemical analysis

During the fermentation of the probiotic beverage, several physic-chemical parameters were examined, including total titratable acidity (TTA), pH, total soluble solids (TSS), and reducing sugars. The TSS content was measured using the index of refraction method described in the study by Pourdarbani *et al.* (2020). To determine TTA, the sample was diluted (1:10) and titrated against 0.1N NaOH, following the method described by Vivek *et al.* (2018). The pH of the beverage was measured by using a pH meter. The glucose concentration during fermentation was assessed by utilizing the DNS method explained by Miller (1959). Additionally, the antioxidant activity of the beverage was measured in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity as described by Herrera-Cazares *et al.* (2021).

2.4 Statistical analysis

All the experiments were repeated three times, and each sample was tested twice. The data obtained were presented as the mean ± S.D. Microsoft Excel, a statistical software program was used to analyze the data.

3. Results

The study investigated the impact of mango pulp and mango probiotic drink on the survival of *L. plantarum*, a probiotic lactic acid bacterium, during a four-week storage period at 10ºC. Additionally, the study monitored the weekly changes in pH, total titratable acidity (TTA), and total soluble solids. Table 1 shows a summary of the effects of the different treatments investigated during 24 h of inoculation. Table 2 shows the effect on different parameters during four weeks of study.

3.1 Growth of *L. plantarum* during mango juice fermentation

The study found that the bacteria used were able to grow well in mango juice without additional nutrients and quickly utilize it for lactic acid production and cell synthesis. The viable cell count of the strain during fermentation is presented in Table 1, and a slight increase in microbial population was observed in the first 24 h of fermentation. This could be due to the difference in culture and fermentation media, resulting in a higher growth rate at the initial stage of fermentation. During long-term storage, there was an increase in the total log count of strain cells. Table 1 displays the variations in acidity, antioxidant activity, and pH of the mango juice throughout the fermentation process. Initially, the pH was recorded as 4.7, which declined to 3.9 after fermentation.

Table 1: Total log count, titratable acidity, pH, and antioxidant activity for the storage period of 24 h

Storage time (h)	Log count (CFU/ml)	Titratable acidity (%)	Sample antioxidant activity (%)
0	8.5 ± 0.3	0.07 ± 0.01	69.5 ± 0.1
4	8.9 ± 0.5	1.09 ± 0.01	71 ± 1
8	9.5 ± 0.28	1.10 ± 0.01	74.05 ± 0.1
12	9.8 ± 0.02	1.12 ± 0.01	80.2 ± 0.1
24	9.9 ± 0.1	1.25 ± 0.03	88.03 ± 0.76

All values are expressed as Mean ± SD (n=3).

Table 2: Total log count, titratable acidity, pH, reducing sugar, and antioxidant activity for the storage period of four weeks

Storage time (weeks)	Log count (CFU/ml)	pH	Titratable acidity (TTA)(%)	Reducing sugar (mg/ml)	Antioxidant activity (%)
0	8.5 ± 0.3	4.7 ± 0.1	1.25 ± 0.01	0.06 ± 0.001	69.5 ± 0.1
1	9.9 ± 0.1	4.0 ± 0.1	1.45 ± 0.02	0.1 ± 0.001	88.03 ± 0.76
2	10.1 ± 0.2	4.0 ± 0.1	1.55 ± 0.01	0.03 ± 0.001	63.4 ± 0.1
3	10.5 ± 0.2	3.9 ± 0.1	1.65 ± 0.15	0.03 ± 0.001	60.31 ± 0.1
4	10.9 ± 0.2	3.1 ± 0.1	1.75 ± 0.01	0.02 ± 0.001	63.4 ± 0.01

All values are expressed as Mean ± SD (n=3).

In this study, we studied the shelf-life of fermented mango drinks by incorporating the *L. plantarum* (NCDC 19) probiotic strain. The current study has shown that *L. plantarum* can withstand the harsh conditions of low pH and high acidity in fermented juices. Additionally, there was a significant difference in the shelf-life of the fermented mango pulp compared to the regular mango pulp.

3.2 Changes in total titratable acidity (TTA) during the storage

The change in acidity during fermentation by *L. plantarum* is observed for the first 24 h after making the mango probiotic. There is an increase in acidity during 24 h (Table 1).

3.3 Substrate consumption during mango juice fermentation

During the fermentation process, there was a decrease in sugar levels, with the initial value of 0.06 mg/ml decreasing to 0.02 mg/ml. Additionally, the pH of the medium decreased while the acidity increased. Although, mango pulp has an initial value of pH 4.7 and lactic acid fermentation of the mango juice has lowered the pH as low as 3.9 in 24 h fermentation.The viable counts of *L. plantarum* were found to gradually decrease during cold storage and remained at 10.9 ± 0.2 CFU/ml after four weeks. Having a substantial quantity of viable lactic acid bacteria in probiotic products is crucial to obtain various health benefits.

3.4 Changes in antioxidant activity in the storage weeks

The DPPH percent inhibition method was used to evaluate the antioxidant activity. The results of various antioxidant tests conducted on extracts obtained through different extraction techniques were presented in Table 1. The extract stored for 24 h exhibited the strongest antioxidant activity with a DPPH % inhibition value of 88.03 ± 0.76.

4. Discussion

Mango is widely liked by the consumer not because of its taste but also of its nutritional and medicinal properties. Nguyen (2020) has reported the high amount of phenol and flavonoid in mango leaves. Mango pulp is established for its anti-inflammatory, neuroprotective, antioxidant, and immunomodulatory activities (Chellammal, 2022**)**.

According to Wattanakul *et al.* (2020), a decrease in the pH level of the growth medium can enhance the maximum growth of viable cell counts.The pH plays a crucial role in determining the growth of viable cell counts, and there is an inverse relationship between pH and viable cell counts. In other words, the higher the pH, the lower the viable cell counts, and vice versa. Reddy *et al.* (2015) investigated the fermentation of mango pulp using probiotic lactic acid bacteria such as *Lactobacillus acidophilus* (MTCC 10307), *Lactobacillus delbrueckii* (MTCC 911), *Lactobacillus plantarum* (MTCC 9511), and *Lactobacillus casei*. They aimed to improve the nutritional value of the mango pulp, and they found that the resulting fermented beverage from the mango pulp had excellent sensory properties.The study showed that *L. plantarum* could survive in fermented juices under conditions of low pH and high acidity. Additionally, the shelf-life of mango pulp and fermented mango pulp was found to be significantly different. The research also investigated the total titratable acidity of the mango probiotic drink during fermentation and found that it slightly increased after 24 h. According to Pereira *et al.* (2011), *L. plantarum* and *Pediococcus pentosaceus* grew well in carrot juice with an initial pH of 6.4 at a temperature of 30^{o}C. During fermentation, the mixed culture showed the highest microbial growth, and the final cell concentration was 9.9 ± 0.1 log (CFU/ml), as reported by Coulibaly *et al.* (2020). It was found that *L. plantarum*, *L. casei*, and *L. delbrueckii* grew rapidly on sterilized cabbage juice without the need for nutrient supplementation, reaching a concentration of almost 8.00 Log CFU/ml after 48 h of fermentation at 30^{o}C (Yoon *et al.,* 2006). As the fermentation progressed, there was a decrease in sugar levels, which could be attributed to the high acidity, low pH, and strong antioxidant activity. To obtain the expected health advantages from probiotic products, it is necessary to ensure that they contain a sufficient quantity of live lactic acid bacteria. However, the decrease in pH of the medium and the accumulation of organic acids due to growth and fermentation have been identified as major factors that can negatively affect the viability of probiotic strain (Reddy *et al.,* 2015).This study shows the elucidation of antioxidant activity which was calculated by DPPH% inhibition. The results for the highest antioxidant activity in terms of DPPH% inhibition observed for 24 h showed similarity with the previous work (Herrera-Cazares *et al.,* 2021). There is a reduction in antioxidant activity was observed during extended storage for four weeks. DPPH inhibition is recommended for the calculation as it is one of the effective ways of calculating the antioxidant activity. The knowledge obtained from this study will hopefully be useful to prepare not only any probiotic drink but also all probiotic products.

5. Conclusion

The findings of this study have shown that *L. plantarum* can survive in mango juice that has been fermented at low pH and with high acidity levels. This suggests that fruit juices could be utilized as a fermentation medium for delivering probiotic LAB to individuals who are lactose intolerant or have allergies to milk-based products. Probiotic fruit beverages are healthy, cost-effective, and free of cholesterol, and may offer better health and nutritional advantages to the public. Through this research, a new probiotic fruit beverage based on mango, which has several health advantages, may be introduced.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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