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Development of fermented probiotic beverage by using cucumber and bottle gourd juice a research paper

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Abstract
Probiotics are living micro-organism, which mainly have several advantages on human's health. Nowadays, demands for non-dairy probiotic beverages are increasing day by day as vegetarianism is becoming popular especially in developed countries, due to the factors such as lactose intolerance and cholesterol content associated with intake of probiotic dairy based products. Cucumber (*Cucumis sativus*) and Bottle gourd (*Lagenaria siceraria*) are a good source of nutrients. In the present study work, with the aim of utilizing cucumber-bottle gourd to develop a fermented beverage, formulated cucumber-bottle gourd were evaluated as a potential substrate for the production of probiotic fermented juice by *Lactobacillus acidophilus.* Numerous factors such as starter culture concentration, cucumber-bottle gourd concentration and fermentation time were optimized on the basis of growth and other physico-chemical parameters such as pH, Total Soluble Solids and Titratable Acidity. Among all the evaluated culture concentration, 10% inoculum size was observed to be most suitable with 16 hr of fermentation time at 37 °C. The value of viable cell count, pH and titrable acidity in cucumber-bottle gourd juice after 16 hr of fermentation was 8.02×10^8 CFU/ml, 3.64 and 1.84% respectively. Furthermore, during the storage period of 28 days at 4 °C, the viable cell count of probiotic bacteria gradually decreased. In addition, change in physico-chemical parameters was observed during cold storage in which pH slightly dropped and TA slightly increased within 4 weeks of storage.

Keywords: Fermented probiotic beverage, cucumber, bottle gourd juice

1. Introduction
Food is a building block of a human body, it plays very crucial role by satisfying hunger, by providing essential nutrients, regulating health, icreasing physical and emotional well-being and also ignoring or decreasing nutrition-related disorders. Moreover these days consumers perception regarding food and health has raised their interest in "Healthy Foods". Nowadays "Functional Foods" supply prominent health benefits on the human body as well as standard nutritional impacts. Functional beverages are very important components of the food industry and also they are known for their nutritional qualities (Chavan *et al*., 2018) [3].
Bioactive segements which includes oligasaccharides, vitamins, dietary fibres, vitamins, minerals and also active "friendly" bacteria, which can be also called as probiotics, are very known category of functional food which helps in improving and regulating the intestinal microflora (Patel, 2016). The nutritionists and health professionals are likely promoting the importance of probiotic foods on human health. Probiotics are found in fermented milk and yoghurt at the market these days. However, nowadays the demands for non- dairy probiotic beverages are increasing day by day as vegetarianism is becoming popular especially in developed countries. In recent years, the demands for non-dairy probiotic product has been increased due to the factors such as lactose intolerance and cholestrol content associated with the intake of fermented dairy products. Due to their wide dispersion and highly nutritional value, vegetables provides an efficient alternative for the processing of probiotic food. Lactic acid fermentation is beneficial in improving the shelf life, safety, nutritrional and sensory characteristics of all types of vegetables. Several *Lactobacillus species* strains have been observed to exhibit an improving health-recovering element which basically includes pathogen resistance, immunodulation and blood cholesterol reduction, which are generally utilised as probiotics. According to the phenomenon, beverages are the upcoming food category in which the crucial bacteria will make an appearance, with fresh fruits and vegetables juices which will become a viable options (Sharma and Mishra, 2013) [16]. Over thousands of years lactic acid bacteria (LAB) have been used in the manufacturing of fermented food products due to their capability to induce different flavours, texture and taste alteration.

The different kinds of antimicrobial compounds produced by these micro-organisms, such as lactic acid, acetic acid, hydrogen peroxide and bacterioxins, are basically known for inhibition of foodborne pathogens and food spoilage micro-organisms, hence prolonging its shelf-life and improving food safety. In addition, Lactic acid bacteria in fermented food products contributes sensory properties to the food such as taste, aroma, texture and simultaneously helps in lowering its Ph and increasing its quality and safety (Singh *et al*., 2017).

Since the beginning of civilization, the fermented food products have been manufactured and consumed and they have long been a crucial part of the humans diet. Fermentation is the process where different micro-organism such as bacteria and their enzymes produces different beneficial changes in food products. The micro-organisms which are involved in the fermentation of food products can be divided into three categories i.e. lactic acid bacteria (LAB) from the genera *Leucoknostoc, Lactobacillus, Streplococcus*. Food substrates, such as meats, fish, dairy, vegetables, soy beans and other legumes, cereals, starchy roots and grapes and other fruits, are utilised for understanding fermentation (Admassie, 2018) [1]. Fermentation of a food product is a very cheap technique for preservation increasing nutritional quality and enhancing sensory properties which is independently discovered worldwide. Fermentation of dairy and non-dairy beverages, grains and other substrates helps in producing health- promoting beverages. In many parts of Asia, Africa, Europe, the Middle East and South America, fermentation of food products are done in a very vast scale. Fermented vegetable juices, rice, honey and fruit beverages were found in pottery jars back in 7000 B.C. The global market, consisting of functional food and beverages, for an illustration, have rose by 1.5 times between 2003 and 2010 and is predicted to expand around €22.8 percent from 2010 to 2014 which then reaches upto €21.7 billion, according to some projections, with the market reaching around €65 billion by 2016 (Marsh *et al*., 2018).

According to recent studies, vegetables juices are highly rich in nutrients, antioxidants and other health promoting substances. Vegetables juice is considered to be an important and prominent part of a healthy diet and thus, it should be consumed on a regular basis. Cucumbers are the one of the most popular fermented vegetables worldwide, especially in United states. Currently, a lot of attention have been given to the distribution and succession of a lactic acid bacteria in cucumbers. On a total average of 47000acres, which is around 550000 metric tonnes of cucumbers are farmed. Cucumbers are likely to be processed into a different types of flavoured whole or sliced into pieces, which are basically consumed at a rate of 3.7 kg per capita per year (Fleming, 1984) [7]. More than above hundreds of vine species producing coiled, climbing tendrils belongs to the cucurbitaceae family. Bottle gourds are among one of the healthiest vegetables which are quite health-promoting for the human's body. Bottle gourds are well known for their medicinal properties among numerous gourds. Bottle gourds juice is highly rich in all of the necessary constituents such as amino acids and all other crucial nutrients which are needed for the well-being of a human body (Palamthodi *et al*., 2019) [11].

2. Materials and Methodology

2.1 Materials

2.1.1 Procurement of raw materials and its storage

The raw materials which were required for the research work includes Cucumber (*Cucumis sativus*) and bottle gourd (*Lagenaria siceraria*). These vegetables were procured from local market of Jalandhar, Punjab.

2.2 Medium preparation

2.2.1 Preparation of MRS broth

As per the manufacture's instruction, MRS broth was prepared by following steps:

I. Lactobacillus MRS broth GM369 (direction: suspend 55.15 grams in 1000ml purified/distilled water) was collected from lab under the surveillance of lab assistance.
II. According to requirement, 5.51g MRS Broth was added to 100 ml of distilled water in a conical flask.
III. Thereafter, it was dissolved on a heating metal (around temperature 80 ℃) by using glass rod.
IV. After that the conical flask containing medium is properly locked by using sterilized cotton plug.
V. For sterilization, MRS broth was kept in a Autoclave at a temperature of 121 ℃, 15psi for 15 minutes.
VI. Autoclaved medium was cooled down to the room temperature.
VII. Finally, 1% (v/v) of filter sterilized (0.22 µm) cysteine HCl was aseptically added to the prepared MRS medium.

2.2.2 Microbial Culture and Media

Lactobacillus acidophilus MTCC-10307 was used as a culture of probiotic strain for this study. This culture was added to the already prepared media (MRS Broth), thereafter it is kept in incubator for 24 hrs at 37 ℃ for the microbial growth of the culture. Lactobacillus acidophilus MTCC-10307 used in this study was procured from the National Dairy Research Institute, Karnal, Haryana, India.

2.2.3 Preparation of seed culture

1. For the preparation of seed culture, around 1% of lyophilized probiotic cultures were added in 100ml of already prepared MRS broth in 250 ml conical flask and the process of revival was done under the Laminar flow cabinet in order to avoid contamination, furthermore, for the growth of primary culture it is kept in incubator at 37 ℃ for 24 hrs.
2. After 24 hrs of incubation, for the formation of secondary seed culture, 10% (v/v) of primary seed culture was transmitted into 100 ml of prepared MRS-broth using micro pipette and sterilized tips was done under to laminar flow cabinet and thereafter, it is again incubated at 37 ℃ for 24 hrs.
3. Then, the process was repeated by transferring (10% v/v) of secondary seed culture into 100ml of MRS broth by using micro pipette and sterilized tips under laminar flow cabinet and thus, it is cultivated at 37 ℃ for 24 hrs in incubator for the growth of tertiary seed culture.

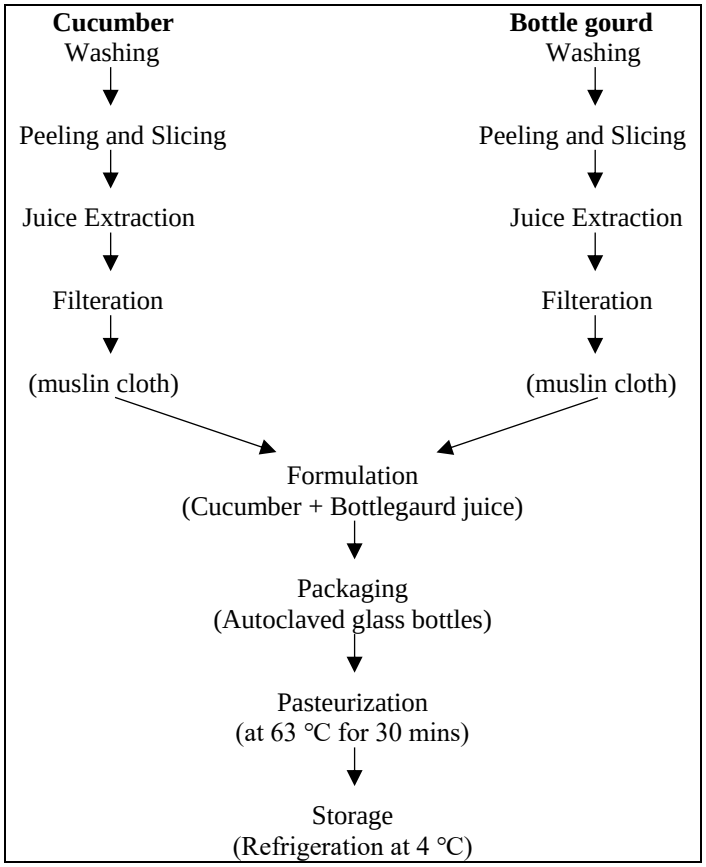
Furthermore, to examine the growth of probiotic culture, around 2 ml of entire cell culture fluid was taken at every 2 hrs from incubation. 600nm was found to be optical density of seed culture.

2.2.4 Preparation of cucumber-bottle gourd formulated juice fermented medium

All the raw cucumbers and bottle gourd were washed properly for the removal of germs and unwanted residues. Then they were peeled and sliced into small pieces separately. After that

juices of cucumber and bottlegourd were extracted separately using automatic juice extractor in two different containers. Then the extracted juices were filtered using muslin cloth to avoid solid particles of pulp. Then the juices of cucumber and bottle gourd were formulated (mixed) in five different combinations (Cucumber: bottle gourd i.e. 20:80, 40:60, 60:40, 80:20, 50:50) using measuring cylinder. Five sterilized autoclaved glass bottles (250ml) were used for containing 200ml formulated juice (different ratio) in each bottles. Finally, all the juice bottles were pasteurized in hot water bath at a temperature of 63 ℃ for 30 mins. After the cooling down at room temperature, all the prepared juice bottles were stored in a refrigerator at 4 ℃.

The sterility of formulated cucumber and bottle gourd juice (fermented medium) was next tested by spreading a tiny amount of onto potato dextrose agar (PDA) to check for fungi and yeast, nutritional agar (NA) to check for bacteria, MRS agar to check for any indigenous microorganisms.



Flow chart 1: Preparation of Cucumber-Bottlegourd Juice

2.2.5 Pasteurization

All the different of cucumber-bottle gourd Juices were subjected to pasteurization at a temperature of 63 ℃ for 30 mins. All the glass bottles containing juice samples were kept in lab scale water bath maintained at the required temperature. Thereafter, when the desired temperature of juice was achieved, it was held at that temperature for the desired time duration. Fresh cucumber-bottle gourd juice was taken as control. Then the pasteurized juice sample were evaluated for physico-chemical, microbial and sensory analysis. All the glass bottles containing pasteurized juice sample were stored in refrigerator at 4 ℃ for almost 28 days.

2.2.6 Inoculum preparation for cucumber and bottlegaurd juice fermentation medium

The probiotic bacterial inoculum was prepared using starter culture (*Lactobacillus acidophilus*) which was cultivated in MRS broth till tertiary seed culture for 24hrs. Thereafter, the entire 100 ml cell culture fluid was taken and divided into two centrifuge tubes of 50 ml each. Futhermore, it were centrifuged at 10,000 rpm for about 20 mins. As a result, in all two centrifuge tubes deposition of cell pellet was found at the bottom of tubes. Cell pellet was collected and washed for two times with sterile saline solutions (0.85% NaCl). The final pellet was picked up using micro pipett and tips and dissolved into cucumber-bottlegaurd juice fermented medium of formulation 60:40 (100ml).

2.2.7 Effect of different inoculum size on fermented medium

Cucumber-bottle gourd juice fermented medium of combination 60:40 (100 ml juice) was inoculated using already prepared probiotic bacteria inoculum with 1% ,5%, and 10% (V/V) seperately. Thereafter, all the glass bottles containing fermented medium was kept in incubator for further fermentation at temperature of 37 ℃ for 16 hrs. Viability of cell counts of probiotic bacteria were found by using direct pour plate method on MRS-agar plates.

2.2.8 Growth profile of probiotic bacteria in cucumber-bottle gourd juice fermented medium

Cucumber-bottle gourd juice fermented medium with the formulation of 60:40 (100ml) was inoculated with probiotic bacterial inoculum (10% V/V) and kept for fermentation in incubator at 37 ℃ for 16h and later it was stored in refrigerator at 4 ℃ for 28 days. To check the growth profile of probiotic bacteria in cucumber-bottlegourd fermented medium, around 4-5 ml of whole cell culture fluid was taken from fermented medium at interval of every 2 hrs in order to check its change in pH and TSS during fermentation period and viability of cell count were obtained by using simple direct pour plate method.

2.3 Sensory analysis of fermented beverage

The formulated cucumber-bottle gourd fermented juice was analysed for sensory evaluation for various characteristics such as appearance, aroma, flavours, taste, texture and overall acceptability. The evaluators were instructed to document their findings on a sensory data sheet using a scale of 0 to3.

Bad-0.
Good-1.
Very good-2.
Excellent-3.

2.4 Physico-chemical analysis of fermented juice

Analysis of fermented cucumber-bottle gourd juice was done based on different physico-chemical parameters such as pH, total soluble solids, titratable acidity.

2.4.1 Total Soluble Solids (T.S.S)

By using a portable digital refractometer, the total soluble solids content of cucumber bottlegourd juice sample was calculated in °Brix.

2.4.2 Titratable acidity
The 20ml sample was used for the titration with 0.1 NaOH to pH 8.2 in the presence of phenolphlathein as an indicator, and the generated lactic acid was measured as the titratable acidity. Titratable acidity was measured in Soxhlet-Henkel degrees (°SH) and it was derived by multiplying the millitre of Naoh used by two, and the volumetric productivity (measured in grammes per litre per hour) was also determined by multiplying the (°SH) by 0.225 and dividing by the growth time (Sharma *et al*., 2017) [15].

2.4.3 PH
The pH of juice sample was measured by using a digital pH meter (Labman) after standardizing it with buffers oh pH 4.0 and 9.0.

2.5 Storage Study
2.5.1 Effect of storage on viability of probiotics
The Cucumber-bottlegourd beverage was stored at 4 ℃ for 28 days, in refrigerator. The assessments of the probiotics cell viability were done at the weekly intervals. The MRS media were used to count the number of yeast and bacterial cultures the direct plate pouring method was used to cultivate 0.1 ml of the diluted test material from each dilution on 20-25ml of autoclaved MRS agar. Thereafter, by using colony counter, all the colonies were counted manually after all plates had been inculated in incubator at 37 ℃ in anaerobic atmosphere for almost 24-48 hours. The viable count cell count was measured in CFU/ml.

2.5.2 Effect of storage on physicho-chemical properties of probiotic beverage
The cucumber-bottlegourd juice was kept in refrigerator at 4 ℃ for 28 days. Probiotic beverage's physicho-chemical properties including pH, Total soluble solid (TSS), Titratable acidity were assessed at weekly intervals to study the effect of storage temperature on microbial metabolism.

3. Result and Discussion
3.1 Optimization of substrate condition
For the optimization of substrate concentration of cucumber-bottle gourd juice, various combination (i.e. 20:80, 40:60, 60:40, 80:20 and 50:50) were utilised. All the different combination juice samples were evaluated on the basis of their sensory characteristics. Among all the combination of substrate, 60:40 ratio juice sample was accepted for the study of research work due to its suitability for various characterstics such as appearance, aroma, flavour, taste, texture and overall acceptability. The numbers of viable cells and change in pH and TA are given in the upcoming tables. It has been found that with the increase in the number of viable cells, the pH of the medium decreases and TA increased. This might be due to the conversion of sugar into lactic acid hence a decrease in pH and increase in TSS. Similar results were observed by Angelov *et al*., (2007) and Sharma *et al*., (2017) [15] during Oats and whey fermentation respectively.

3.2 Growth pattern of probiotic bacterial colonies on MRS-agar plate

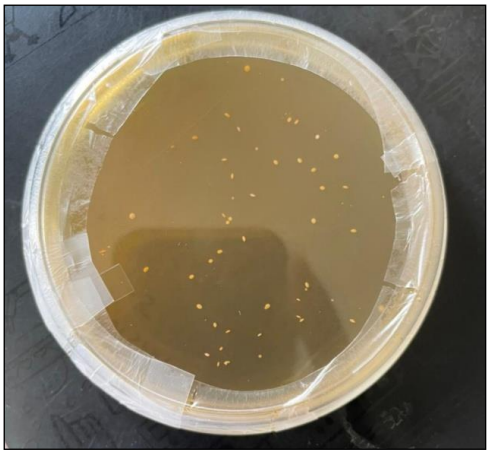


Fig 1: Growth of probiotic strain on MRS-agar plate

Colonies were discovered to have marphological similarities to *lactobacillus acidophilus* based on the formation of probiotic bacterial colonies on MRS-agar plates.

3.3 Evaluation of pasteurized cucumber-Bottlegourd juice fermented medium
The glass bottles containing 60:40 ratio of cucumber-bottlegourd juice sample was examined for its sterility and the existence of any degrading and the existence of any degrading microorganism after the process of pasteurization of fermentation media. The enhancement of shelf life of fermented beverage can be drastically effected by the existence of unnecessary and unwanted pathogens. As a result, the absence of microbial growth on MRS agar plates demonstrated that the pasteurized cucumber-bottlegourd fermented media had been completely sterilized.

3.4 Preparation of seed culture for Cucumber-Bottlegourd juice fermentation medium
According to guidelines for probiotic products, a minimum level of 6 log CFU/ml is required for better effectiveness in managing the positive effects (Pereira *et al*., 2013) [12]. As a result, whole culture cell fluids from the tertiary seed culture were taken at intervals of 10-12 hours (9.00 log CFU/ml) and fermentation continued for 10-12 hours. Thereafter, the process of fermentation has been gradually declined in order to produce probiotic products that contained at least 10^6-10^7CFU/ml of live bacteria (Khurana and Kanawajia, 2007) [8]. Futhermore, cell culture fluid was centrifuged at 10,000 rpm for about 20 mins and cell pellets were then washed with 0.85 percent Nacl for the removal of media from the fermented medium before being injected with cells in cucumber-bottlegourd fermented medium.

3.5 Optimization of inoculum size

The cucumber bottlegourd fermented medium was inoculated with the starter culture *Lactobacillus acidophilus* (1,5 and 10%) aiming to obtain the required level of viable cells as per the probiotic requirement (10^6-10^8 CFU/ml) and which was incubated for 16h at 37 ℃. Probiotic bacteria were observed to grow in all evaluated medium composition with varying inocuum sizes after 16 hr of static fermentation when each setup was exposed to a viable cells count utilising the direct pour plate method on MRS agar plates. With an inoculum size of 10% and a 16 hr incubation period, the probiotic products desirable qualities, such as a viable count and pH were obtained; however inoculum sizes of 1 and 5 percent did not produce the required level of viable count and pH. Probiotic bacterial growth was its highest in cucumber-bottlegourd fermentation medium with (4.75×10^6 CFU/ml) after 16hr fermentation when inoculated with 10% culture (Table 1). In contrast, the microbial growth in the cucumber-bottlegourd fermented medium was minimum when it was injected with 1% and 5% inoculums. The pH of fermentation medium, which contains 60:40 juice sample, 5.58 falling within the targeted pH range 3.5 to 5.8, while in case of 1% and 5% inoculum size, microbial growth and pH was not up to the desired level. Therefore, Cucumber-bottlegourd fermented medium containing 60:40 ratio and 10% inoculum size was used for further studies.

Probiotic viability and their eventual population in substrates are known to be significantly influenced by inoculum size and fermentation time. Additionally, it has been discovered that inoculum sizes, particularly in the food processing industry, are related to the sensory qualities of finished items, which affect how consumers perceive them and their eventual marketability. An ideal probiotic population in the finished product is the desired result of the processing of probiotic foods. Wardani *et al*. (2017) [18] examined the impact of different inoculum sizes (1, 3, 5 and 10 percent) on *Lactobacillus plantarum* Dad 13's ability to produce organic acids in milk. According to the study, acid generation time significantly decreased as inoculum quantity increased. Chen *et al*. (2016) [4] investigated the effects of *L. casei* and *L. acidophilus* inoculum sizes on the sensory qualities, viable count, acidity, and pH of fermented milk. For each of the two *Lactobacillus* species, inoculum sizes of 1, 3, 5, 7 and 9 percent were utilised and samples were taken out for analysis at 1.5, 3.0, 4.5 and 6.0 h, respectively. In terms of the desirable characteristics looked at, 7 percent inoculum size for both bacteria was found to be exceptional. The 7 percent inoculum size was the most liked according to the sensory evaluation that included taste, smell, colour and comprehensive review. A total viable count of 2.20×10^9 cfu/mL and 1.69×10^9 cfu/mL for *L. acidophilus and L. casei*, respectively, were recorded at the ideal inoculum size and fermentation period of 4.5 h.

Table 1: Effect of inoculum size on growth of probiotic bacteria

Inoculum size	Sample (CFU/ml)	pH
1	2.7×10^6	6.2
5	3.8×10^7	5.98
10	4.28×10^7	5.58

3.6 Growth profile of *Lactobacillus acidophilus* in fermenting medium

In the cucmber-bottlegourd medium the growth of *Lactobacillus acidophilus* was static. The optimised medium cucumber-bottlegourd fermentation medium with the formulation of ratio 60:40 inoculated with 10% inoculum at 37 ℃ for 16hr and their after it was stored at for 28 days at 4 ℃. At every 2 hours of fermentation, whole cell culture fluid was with drawn to analysed the viable cell count using direct pour plate method on MRS agar plate and to monitor the change in pH of the medium during fermentation.

The growth of probiotic bacterial population gradually increased until 16hr of fermentation (8.02×10^8 CFU/ml) as shown in figure 2 and the pH of the fermented juice dropped from 5.70-3.64 indicating that the probiotic bacteria were actively growing and producing lactic acid (Figure3). Because probiotic microbes are pH sensitive, the incubation period of 16 hours has been stopped and fermented medium was refrigerated at 4 ℃ for 28 days. The viability of probiotic bacteria diminishes at an acidic pH, or less than 3.6, as a result of the acidification of the cytoplasm and increased energy requirements for maintaining intracellular pH, which presents enzymatic process and affects the proliferation of the bacteria (Sharma *et al*., 2017) [15]. Due to the need of a sufficient quantity of viable bacteria (i.e. greater than 6 log10CFU/g of product), which provides several health promoting benefits by intake of probiotic beverages but the loss of probiotic bacterial viability also affects its functioning (Ying *et al*., 2012) [19].

Table 2: Growth profile of *Lactobacillus acidophilus* in cucumber-bottlegourd fermented medium

Time (hour)	Viable count (log cfu/ml)	pH
0	4.28±0.10	5.70
2	4.75±0.08	5.58
4	5.57±0.17	5.34
6	5.97±0.17	5.02
8	6.53±0.08	4.76
10	6.82±0.17	4.49
12	7.13±0.10	4.12
14	7.47±0.17	3.80
16	8.12±0.17	3.64

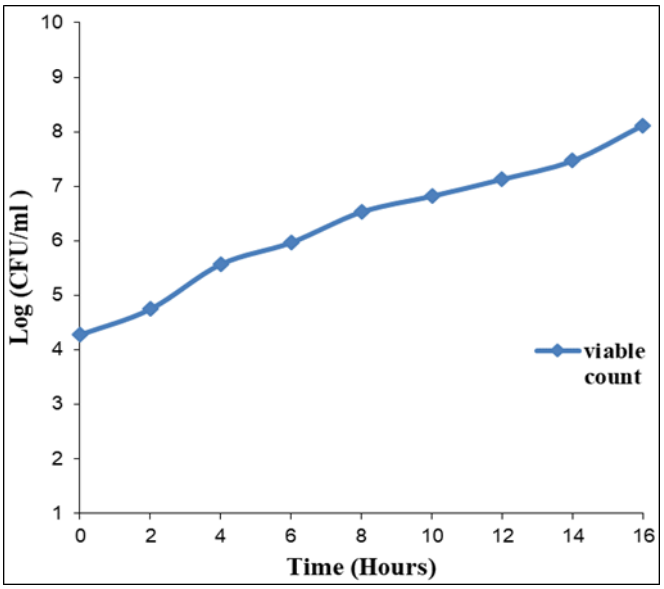


Fig 2: Growth curve of probiotic cultures in fermented juice medium

3.7 Effect of fermentation on physico-chemical analysis

For the effecient outcomes, the microbial fermentation was carried out under optimal condition (i.e. inoculum size10% and incubation time: 16 hr). Table 3 displays the effect of fermentation on the physical chemical characterstics of cucumber-bottlegourd juice such as change in pH, Titratable acidity (% lactic acid v/v) and total soluble solids TSS (°Brix). At initial stage, pH and temperature of the fermentation medium have a direct impact on the development and metabolism of microbes. Decreased in pH (5.70-3.64) was observed during the fermentation period of 16hr at 37 ℃. The fermentation process potential contribution to the pH drop could be the conversion of sugars to organic acid like lactic and acetic acid during fermentation (Dimitroriski *et al*., 2021) [5]. Initially, the pH of fermented juice was observed to around 5.70 which gradually decreased to 5.58 after 2hr. At the fermenting period of 14hr and 16hr, there was slightly change in pH was found around 3.80 and 3.64 respectively.

In contrast with pH, the initial titratable acidity (expressed in % lactic acid v/v) was found to be 0.24% which further increased to 0.39% after the fermentation period of 2 hr. 1.84% of titratable acidity was observed after 16hr of fermentation of cucumber-bottlegourd juice which was further stored for 28 days at temperature 4 ℃.

It was found that the total soluble solids (°Brix) concentration was between 3.8 to 3.4 °Brix during 16 hr fermentation period of cucumber-bottlegourd juice. Negligible changes were observed in TSS values during the incubation period. According to studies, the addition of more sugary substances, which can raised the value of TSS, such change in the value of TSS would result in alteration in sugar content owing to metabolism by fermenting bacteria. Although the value of TSS is checked for quality assessment, the TSS study shows drop in value of TSS which further results in a reduction in sugar concentration due to which fermented cucumber-bottle gourd juice is preferable more than unfermented preparation for pateints with hyperglycemia or other health related problems associated with the metabolic syndrome. (Managa *et al*., 2021) [9].

Table 3: Effect of fermentation on change in pH, Titrable acidity and TSS

Time (hr)	pH	Titratable acidity (% lactic acid v/v)	TSS (°Brix)
0	5.70	0.24	3.8
2	5.58	0.39	3.7
4	5.34	0.61	3.7
6	5.02	0.87	3.6
8	4.76	1.12	3.6
10	4.49	1.43	3.5
12	4.12	1.60	3.5
14	3.80	1.78	3.4
16	3.64	1.84	3.4

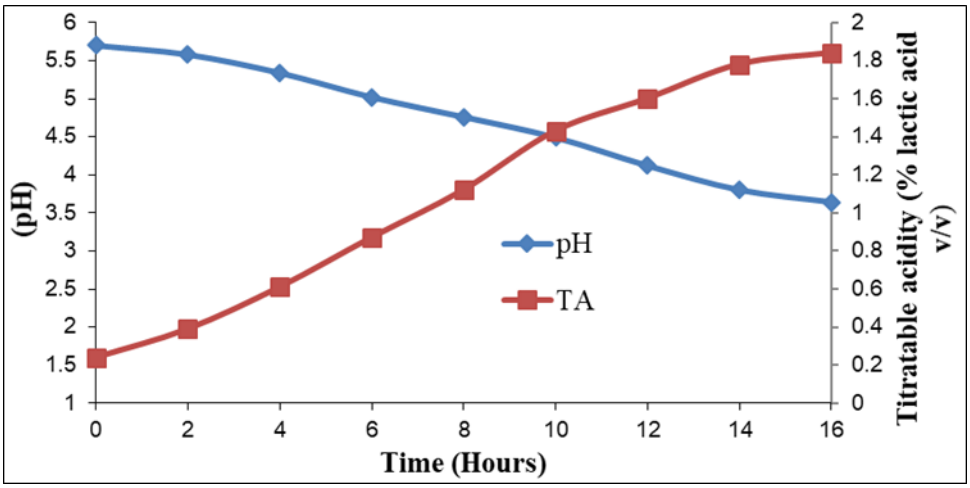


Fig 3: Change in pH and Titratable acidity of fermented juice medium

3.8 Effect of storage on viability of probiotics

The probiotic bacteria chosen for commercial purpose must maintain their viability and functional activity during the enhancement of shelf-life of beverage. Thus, the viability of probiotic bacteria, which basically rely on the amount of oxygen in the beverage, oxygen permeation of the package, and incubation time and the temperature of storage, is the most essential element during refrigerated storage (Shah, 2000) [13]. Table 4. Demonstrate the variation in viable count of probiotic microbes over the course of storage. The probiotic culture were able to survive in the cucumber-bottlegourd juice for 28 days at 4 ℃. The initial viable count of probiotic bacteria was 8.12×10^7 CFU/ml. Over the time course of probiotic bacteria was 8.12×10^7 CFU/ml. Over the

time course of storage, the viable count significantly decreased week by week. The microbial population of Lactobacillus acidophilus gradually decreased to 5.37 $\times 10^6$ CFU/ml in the last week of 28 days of storage. One of the most significant elements affecting the viability of probiotics is temperature, which further enhances the mortality effect impact of organic acids. Lactic acid bacteria cell wall is made up of saturated, unsaturated and cyclic carbon chains. Factor like temperature, pH, Nacl concentration and medium composition are responsible for variation in cell wall. Acidic conditions will allow the occurrence of synthesis of linoleic and oleic acids. When exposed to hostile situation when in an acidic atmosphere during storage at refrigerator temperature these acids will absorb hydrogen, increasing the permeability of porton in membranes. As a result viability of probiotic bacteria in product will increase. According to Sheehan *et al*., (2007) [17], from the standpoint of consumer's health benefits, the selected probiotic cultures must maintain their viability and functionality during the product storage period.

Table 4: Effect of storage on viability of probiotic cultures in cucumber-bottlegourd beverage

Time (weeks)	Viable count (Log cfu/ml)
0	8.12±0.18
1	7.56±0.08
2	7.12±0.12
3	6.41±0.17
4	5.37±0.18

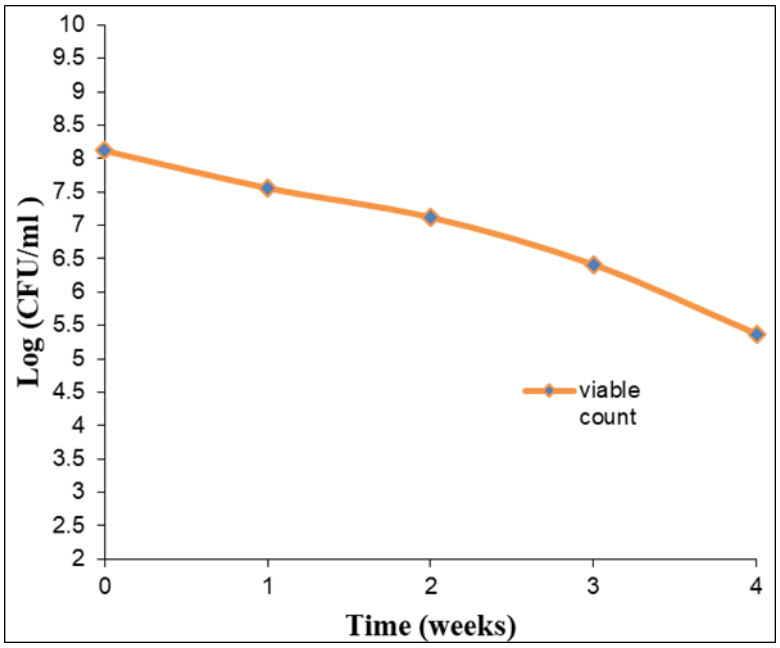


Fig 4: Effect of storage on viable cell counts

3.9 Effect of storage on physicochemical properties of probiotic beverage

The pH and titratable acidity (expressed in % lactic acid v/v) demonstrated a reversal relationship with respect to one another. As a result, over the period of storage i.e. 28 days at 4 ℃, pH gradually dropped whereas the titratable acidity dramatically rose. The cucumber-bottlegourd had 1.84% titratable acidity after 16 hr fermentation period. Within the period of 7 days, the amount of lactic acid considerably increased to 1.86%. After 4 weeks of storage at 4 ℃, the value of titrable acidity was increased 1.9%. On the other hands, the pH of probiotic juice slightly decreased from 3.64-3.62 in the first week of storage. Thereafter, the pH had dropped to 3.58 in the last week of storage at 4 ℃. Initially the value of total soluble solids was around 3.4 °Brix. During the time of storage the value of total soluble solids of cucumber-bottlegourd juice dropped from 3.4-3.2 with the span of storage period for 28 days at 4 ℃. Negligible changes was observed in the value of TSS within 4 weeks of storage.

Probiotic bacteria may have metabolized the simple sugars which are mostly present in the beverages, further results in releasing of small amount of organic acid, because of this reason, drop in the value of TSS and pH and rise in value of titratable acidity have been observed (Shah *et al*., 2010) [14]. The production of enzymes results in hydrolysis of the juice sugars from dead bacteria (Ding and Shah, 2008) [6]. According to several writers, the ability to tolerate acid is a critical probiotic feature for survival during food medium fermentation.

Table 5: Effect of storage on pH, Titratable acidity and TSS of Probiotic beverage

Time (weeks)	pH	Titratable acidity (%lactic acid)	TSS (°Brix)
0	3.64	1.84	3.4
1	3.62	1.86	3.4
2	3.6	1.88	3.3
3	3.59	1.89	3.2
4	3.58	1.9	3.2

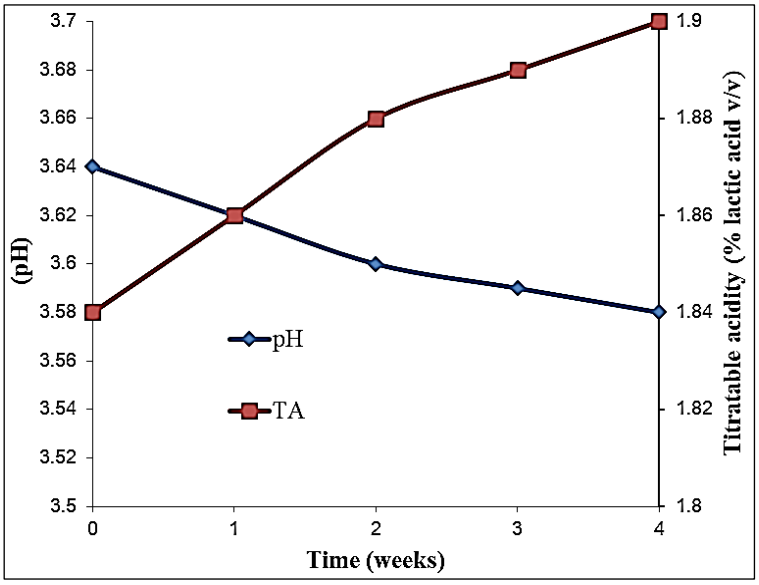


Fig 4: Change in pH and TA during storage period

4. Conclusion

The present investigation focuses on the development of probiotic beverahe which imposes potential health benefits of Cucumber (*Cucumis sativus*) and Bottle gourd juice (*Lageneria siceraria*). For achieving this, the probiotic culture of *L. acidophilus* were used as a starter culture for the fermentation of beverage for preparation of a functional food. The juice was extracted by formulating cucumber and bottle gourd juice together at different ratio (i.e. 20:80, 40:60, 60:40, 80:20 and 50:50). Several factors such as inoculum size, fermentation time, concentration, pH, TSS and TA were optimized. Required viable cell count i.e. ≥ 6 log CFU/ml, pH in the range of 3.6-5.8 was found with 10% culture inoculum after 16 h of incubation period at 37 ℃. Cell viability was found to decrease during cold storage at 4 ℃ for 28 days.

So, one of the forthcoming health beverages could be the produced probiotic beverage. Growing interest in non-dairy probiotic products is a result of the expanding vegan lifestyle trend, lactose intolerance problems, and demand for low-fat and low-cholesterol diets. Thus, a probiotic beverage made from cucumber and bottle gourd can easily be marketed to appeal to consumers of all ages. Finally, it can be said that the processing technique for making probiotic beverages based on cucumber and bottle gourd juice is techno economically viable and hence may be used commercially. It will also be advantageous to the user because it has nutritional and medicinal properties.

5. References

1. Admassie M. A Review on Food Fermentation and the Biotechnology of Lactic Acid Bacteria. World Journal of Food science and Technology. 2018;2(1):19-24. Doi: 10.11648/j.wjfst.2018201.13.
2. Angelov A, Gotcheva V, Kuncheva R, Hristovoza T. Development of a new oat-based probiotic drink. Int J Food Microbiol. 2006;112:75-80.
3. Chavan M, Gat Y, Harmalkar M, Waghmare R. Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume. LWT-Food Science and Technology. 2018;91:339-344.
4. Chen H, Bao C, Wang C. Response surface methodology for optimizing fermentation conditions of goat yogurt with *Bifidobacterium bifidum* and *Lactobacillus casei*. Emir J Food and Agric, 2016, 547-553.
5. Dimitrovski D, Dimitrovska-Vetadjoka M, Hristov H, Doneva-Shapceska D. Developing probiotic pumpkin juice by fermentation with commercial probiotic strain *Lactobacillus casei* 431. J Food Process Preser. 2021;45(3):e15-245.
6. Ding WK, Shah NP. Survival of Free and Microencapsulated Probiotic Bacteria in Orange and Apple Juices. International Food Research Journal. 2008;15:219-232.
7. Fleming HP. Development in cucumber fermentation. Journal of chemical Technology and Biotechnology. Biotechnology. 1984;34(4):241-252. https://doi.org/10.1002/jctb.280340404
8. Khurana HK, Kanawajia SK. Recent trends in development of fermented milks. Curr. Nutr. Food Sc., 2007.
9. Managa MG, Akinola SA, Remize F, Garcia C, Sivakumar D. Physicochemical Parameters and Bioaccessibility of Lactic Acid Bacteria Fermented Chayote Leaf (*Sechium edule*) and Pineapple (*Ananas comosus*) Smoothies. Front Nutr. 2021;8:120.
10. Marsh A, Hill C, Ross RP, Cotter Paul. Fermented beverages with health-promoting potential: Past and future perspectives. Trends in Food Science & Technology, 2014, 38. 10.1016/j.tifs.2014.05.002.
11. Palamthodi S, Kadam D, Lele SS. Physicochemical and functional properties of ash gourd/bottle gourd beverages blended with jamun. Journal of food science and technology. 2019;56(1):473-482. https://doi.org/10.1007/s13197-018-3509-z
12. Pereira AL, Almeida F, De Jesus AL, Da Costa JM. Storage Stability and Acceptance of Probiotic Beverage from Cashew Apple Juice. Food and Bioprocess Technology, 2013, 6. 10.1007/s11947-012-1032-1.
13. Shah NP. Probiotic bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science. 2000;83:894-907.
14. Shah NP, Ding WK, Fallourd MJ, Leyer G. Improving the Stability of Probiotic Bacteria in Model Fruit Juices using Vitamins and Antioxidants. Journal of Food Science. 2010;75:278-282.

15. Sharma P, Gat Y, Trivedi N. Development of functional fermented whey-oat based product using probiotic bacteria. 3 Boitech, 2017, 7(4).
16. Sharma V, Mishra HN. Fermentation of vegetables juice mixture by probiotic lactic acid bacteria. Nutafoods. 2013;12:17-22. https://doi.org/10.1007/S13749-012-0050-y.
17. Sheehan VM, Ross P, Fitzgerald GF. Assessing the Acid Tolerance and the Technological Robustness of Probiotic Cultures for Fortification in Fruit Juices. Innovative Food Science and Emerging Technologies. 2007;8:279-284.
18. Wardani SK, Cahyanto MN, Rahayu ES, Utami T. The effect of inoculum size and incubation temperature on cell growth, acid production and curd formation during milk fermentation by *Lactobacillus plantarum* Dad 13. Int Food Res J. 2017;24(3):921-926.
19. Ying D, Schwander S, Weerakkody R, Sanguansri L, Gantenbein-Demarchic C, Augustina MA. Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. J Funct. Foods. 2012;5:98-105.