



Evaluation of physicochemical properties and sensory acceptability of apple, carrot, beetroot mixed juice as probiotic juice

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ABSTRACT

In the recent years, growing interest has been seen in the production of functional foods that promote health and prevent disease. Probiotics are useful to human health in many ways. The varieties of probiotics that are now on the market are mostly dairy based, indicating that people with allergies to milk proteins or lactose intolerance and consumers who are vegans can not take them. Vegetables and fruits provide excellent alternatives for the manufacturing of probiotic foods due to their wide availability and high nutritional content. The apple, carrot, and beetroot were used as raw materials to make a probiotic mixed juice while looking for substitute food matrices.

The purpose of this study was to create healthful probiotic mixed juice blends of apple juice and carrot and beetroot juice. The juices of the apple, carrot, and beetroot were mixed together to create a blended juice that was fermented for 48 h at 37° C after the inoculation of probiotics and kept in a glass container in the refrigerator at 4° C for 21 days. The study was undertaken by comparative examined the analysis of physicochemical characteristics such as pH, total soluble solids, titratable acidity, moisture content, ash content, total solids, fat content, protein and sensory evaluation of fresh mixed juice and probiotic mixed juice. The changes in pH, total soluble solids, titratable acidity and cell viability of probiotic mixed juice were examined during the fermentation at 37° C and storage at 4° C for 21 days. The pH of fermented mixed juice reduced from 5.02 to 3.95 after fermentation of 48 h and maintained for up to 14 days of storage and gradually increased to 4.01 after 21 days of storage. The probiotic count reached to 10⁶ CFU/ml after fermentation of 48 h but gradually started losing their viability after the two weeks of storage. In the sensory testing, the fresh mixed juice earned the greatest score given by the panelists in comparison to probiotic juice. But according to health aspect the probiotic mixed juice after the storage of 14 days at 4°C, scored higher than the probiotic juice of 21 days of storage. This product is extremely helpful for the people who vegan diet and are intolerant to lactose and unable to consume probiotics through milk or milk products. And it is advised to be used with the 14 days.

Keywords: Apple, Carrot, Beetroot, Probiotic juice, Fruit, Vegetable, Non dairy products, Vegan, Physicochemical characteristics

1. INTRODUCTION

In today's lives, food has a variety of purposes in our lives, such as maintaining the health of our bodies and minds, fulfilling hunger, supplying the nutrients we need, improving our overall wellness, and preventing illnesses due to insufficient nutrition (**Perricone et al., 2015**). Consumers have now getting more aware of the connection between diet preferences and overall health in the last several years. Consumers are actively seeking out healthier items that contain better nutritional qualities and particular ingredients in order to avoid health problems, improve their overall wellness, and extend their lives. As a result of rising knowledge,

"functional foods" and "nutraceuticals," which are dietary products intended to improve consumer health and lower their chance of developing chronic illnesses, have become more popular (Manoj et al., 2023). Foods that are high in nutrition, offer health benefits, prevent disease, and promote health are now more widely accepted by the market and make successful advertising or branding tools. As a result, functional foods have become more popular. These include a variety of ingredients such as dietary fiber, vitamins, minerals, probiotics, and prebiotics (Nematollahi et al., 2016).

Probiotics are known as live microorganisms (Guarner and Schaafsma, 1998), that improve the microbial balance in the gut and benefit the host (Vasudha and Mishra, 2013). Probiotics are dietary supplements that comprise live strains of microorganisms. These strains may colonize the human digestive system and remain there for an extended period of time, which allows them to have positive impacts on the host's physiology. These results involve enhancements to general health and overall well-being (Jan et al., 2023). Probiotics have been shown to have a major impact on respiratory, digestive, and immune system processes as well as to significantly reduce the symptoms of infectious diseases.

Health professionals are increasingly promoting the positive effects of food that have been supplemented with live microorganisms on human health, especially for children and other high-risk populations, particularly in milk products (Hotel and Cordoba, 2015) and plant based products as well. In order to add probiotic bacteria to food for commercial or domestic use, they are often accessible as culture concentrates in dried or deep freeze form (Tripathi and Giri, 2014). The food industry has recently been working on producing additional food matrices that are appropriate for this use. Considering fruit and vegetable juices serving as an effective carrier for probiotic microorganisms, the development of probiotic beverages based on vegetables and fruits may represent a compromise choice (Zuntar et al., 2020).

Probiotic microorganisms are primarily added to the dairy products like yogurt and fermented milk and there are availability of limited plant based probiotic food alternatives. Customers' preferences are shifting toward healthier options as a result of growing awareness of milk's allergic properties as well as the high cholesterol and fat content in the milk. Although the market for products made from plants has been expanding more rapidly in recent years. Probiotics have become more and more popular in fruit and vegetable juices (Mojikon et al., 2022).

Since approximately seventy percent of people all across the world suffer from lactose intolerance, the development of plant-based probiotic products enables individuals who are lactose intolerant, allergic to milk proteins, have high cholesterol, are strict vegetarians, or live in areas without access to dairy products to consume these health-promoting microorganisms (Pimentel et al., 2019).

A significant portion of the consumer population regularly and devotedly consumes fruit juice because the fruit is perceived as being a healthful food. Fruits and vegetables are extremely valuable foods because their contents are rich in carbs, minerals, vitamins, dietary fiber, and antioxidants that support the body's alkaline reserve development, maintenance, and repair (Panghal et al., 2017). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) set a recommended daily intake of fruits and vegetables to avoid chronic diseases such as coronary heart disease, hypertension, and stroke risk.

Most juices are manufactured from real fruits, but a small percentage are made from esters that have the flavor of the fruit the manufacturer is interested in marketing. One fruit or a variety of fruits and vegetables can be used to make juices. The fruit contains juice, which can be prepared or extracted by mechanically pressing the fruit to release the juice without using heat. Fruit juices must be very high quality because they go through several processes in the manufacturing and production phases. Fruit juice production and processing are complex, quiring a deeper understanding of their physico-chemical characteristics (Gbarakoro et al., 2020).

Additionally, there have been suggestions for new products containing probiotic strains that are based on fruits, vegetables, soybeans, and cereals. Fruit juices in particular have been characterized as a unique and acceptable medium for probiotics due to their appropriate nutrient content (Luckow et al., 2006). Fruits and vegetable juices does not contain lactose, an allergen to milk that some people are intolerant to and can not consume, like certain other foods do. It is therefore crucial to evaluate the sensory characteristics of the probiotic organisms in fruit and vegetable juice systems from a business standpoint (Luckow and Delahunty, 2004).

Given that some consumers may develop soy allergies even after using items made from soybeans. In addition, companies have entered the dairy-free and vegan drink market in response to environmental, animal, and vegetarian advocacy as well as lactose intolerance. Recipes for probiotic drinks made with fruits and vegetables must be created in order to meet market demand for dairy alternatives and vegan beverages (Cordle, 2004).

In the research of **Nguyen et al., 2019** fermentation of pineapple juice were conducted with probiotic bacteria, specifically *Lactobacillus* and *Bifidobacterium* strains, and observed changes in beverage properties during storage. They found that all strains grew well on pineapple juice without added nutrients. *Lactobacilli* exceeded 5×10^9 CFU/ml, while bifidobacteria reached 10^9 CFU/ml after 24 hours. *L. plantarum* 299V showed the highest productivity. The ratios of lactic acids to acetic acids varied among strains. Probiotic supplementation influenced lactic and acetic acid concentrations. Fructose was the preferred sugar for both bacteria. Total phenolic content and antioxidant capacity increased slightly during fermentation but decreased during storage. After two months, probiotic viability declined after exposure to pepsin and bile salts. This research suggests the potential for developing probiotic pineapple juice.

Shafiya R et al., 2016 aimed to create a non dairy probiotic drink that is non dairy and may be consumed by individuals who are intolerant to lactose, follow a vegetarian diet, or have other health concerns. Carrot juice's usefulness for producing probiotic food containing *Lactobacillus acidophilus*, *Lactobacillus plantarium*, *Lactobacillus casei*, and *Bifidum longum* was explored while searching for alternative probiotic carriers. When compared to fresh carrot juice, the probiotic juice's approximate composition revealed a higher protein concentration and a lower carbohydrate content. The effect of independent factors (pH and temperature) on response parameters (biomass and cell viability) was examined using response surface methodology (RSM). The results of the statistical research showed that the ideal pH of probiotic carrot juice.

Panghal et al., 2017 focused on developing a non dairy probiotic drink using beetroot juice. They studies on the creation of a probiotic beetroot drink had the objective to make a non dairy probiotic drink. Probiotic potential was assessed using *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus delbrueckii*. At pH 6.5 and the ideal fermentation temperature of 37°C, a probiotic drink was made. In due course, the sugar content and pH both gradually decreased. Comparing the probiotic drink to the fresh juice sample, total phenols, flavonoids, and antioxidant effectiveness were higher. According to a study, beetroot drink, which is cholesterol-free and packed with health-promoting ingredients, is a great way to consume probiotics without dairy.

The objectives of the study are to determine the viability of probiotic strain, demonstrate its stability during fermentation in blended probiotic drinks, determine the optimal parameters to maximize probiotic growth and metabolic activities, study the physio-chemical, nutritional properties, and shelf life of the final products, to determine consumer acceptability and analyze the drink's effects on human health and well-being.

Probiotic

The addition of probiotics can frequently boost the value of many foods available on the market (**Zuntar et al., 2020**). Customers who consider themselves to be generally healthy use the probiotic supplements. Many probiotic products are used by consumers who regard themselves as being otherwise healthy. They do this because they feel that probiotics will help them maintain their current level of health and well-being and may lower their long-term risk of developing heart, renal, pulmonary, and intestinal disorders (**Morelli, 2013**).

Probiotic microorganism

Lactobacillus sp.

It has been discovered that *Lactobacilli* enhance the immune system's ability to produce interferon alpha, particularly in the gut immune system. Although they produce compounds that resemble bacteriocin, lactic acid bacteria also have the ability to inhibit a variety of pathogenic gram negative bacteria (**Chateau et al., 1993**). There is another species called *Lactobacillus acidophilus*, and there are strains of it that can withstand the harsh conditions of humans and even make their way through and colonize the entire digestive tract. *Pseudomonas sp.*, *Salmonella sp.*, *Staphylococcus sp.*, and other gram positive and gram negative bacteria are

all inhibited by the antimicrobials produced by *Lactobacillus acidophilus*, which produces acidolin, acidophilin, and lectincin B (Gomez Gil et al., 1998).

Bifidobacterium sp.

Vitamins including riboflavin, thiamine, vitamin B6, and vitamin K, as well as associated bioactive compounds like folic acid, niacin, and pyridoxine, can be synthesized and produced by *Bifidobacteria*. Probiotic formulations containing *Bifidobacterium* include encapsulated bacterial cells, co-encapsulated cells with prebiotics, and single bacterial strains in conjunction with other probiotic microbes (Sharma et al., 2021). The flora of breastfed newborns was shown to be dominated by *Bifidobacterium sp.*, but formula-fed infants showed no signs of this bacteria. Furthermore, the bacteria are resistant to the conditions seen in the human stomach. Consequently, probiotics containing these bacteria are also employed. The species that are employed are *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, and *Bifidobacterium infantis* (Gomez Gil et al., 1998).

Streptococcus sp.

Streptococcus thermophilus is a Gram positive bacteria that is a member of the order Lactobacillales, family Streptococcaceae, and phylum Firmicutes. It is a member of the clade of lactic acid bacteria, which also includes species from the genera *Weissella*, *Tetragenococcus*, *Oenococcus*, *Pediococcus*, *Leuostoc*, *Enterococcus*, and *Lactobacillus* (Sharma et al., 2014). *S. thermophilus* is a common food ingredient that can be found in many different fermented dishes. Moreover, *S. thermophilus* expresses a cell envelope proteinase and produces certain amino acids (Tabora et al., 2024).

Fruits and vegetables

A diet rich in micronutrients, low in calories, and diversified in flavor, color, and taste is made possible through the fruits and vegetables (Sachdeva et al., 2013). They are rich in a diverse range of vitamins, minerals, phytochemicals, bioactives, and antioxidant components. Phenolic substances, among other phytochemicals, are thought to be good for human health because they reduce oxidative stress and limit macromolecular oxidation, which lowers the risk of degenerative diseases (Vasudha and Mishra, 2013). Polysaccharides, cellulose, oligosaccharides, and hemicellulose are the main constituents of fruit fibers. The benefits of fiber on the health are widely known. Increasing dietary fiber intake has been linked to preventing diabetes, promoting good intestinal function, and reducing the risk of obesity and some malignancies (Manoj et al., 2023).

Apple (*Malus Pumila*): Apples also known by scientific name *Malus Pumila*, a member of the Rosaceae Family, they are among the most significant fruits cultivated in the Sucea region. Fresh juice from apple fruits is ingested directly; however, the fruit can also be used to flavor paste, dried fruit, jellies, and jam, as well as soft beverages (El-Dakak et al., 2017). Apples include phenolic substances like flavonoids, which are important sources of antioxidants for diets. By raising the release of endothelial nitric oxide (NO) and causing vasodilation, flavonoids lower the risk of cardiovascular illnesses (Khoo et al., 2010). Apples are a fruit of interest due to their excellent nutritional content and diverse range of bioactive components. They are also relatively affordable, adaptable, and accessible. Fewer studies have examined whether apples consumed in other forms such as apple juice, pomace, cider, vinegar, and others had the same positive health impacts as whole apples, despite the fact that consumption of apples has been linked to a number of favorable health outcomes.

Beetroot (*Beta vulgaris*): Beetroot, sometimes known as red beet, refers to a variety of edible taproot cultivars and is a cultivated version of *Beta vulgaris* subspecies *vulgaris* (*conditiva*). *Beta vulgaris*, or beetroot, is a good source of antioxidant substances phenolics, minerals, vitamins and dietary fibers. The most important biomolecules and micronutrients found in the beetroot included phenolic compounds, carotenoids, betalain, and vitamins and minerals such as sodium, potassium, magnesium, vitamin C, and betanin. Although they have a high concentration of functional components with anti-inflammatory, antioxidant, and anticarcinogenic potential, they may be relevant to human health (Bianchi et al., 2021). The presence of geosmin, which gives beetroots their distinctive earthy flavor, has a detrimental effect on the sensory qualities of food products that

contain beetroot juice, regardless of the fact that beetroot juice has many health benefits. A class of secondary plant metabolites called betalains, which are phenolic, are highly concentrated in beetroots and give them their deep red color. While betalains are utilized by the food industry as natural colorants, they are also gaining interest because of their potential health advantages for people, particularly for their anti-inflammatory, antioxidant properties and chemo preventive effects (**Panghal et al., 2017**); (**Bianchi et al., 2021**).

Carrot (*Daucus carota*): The carrot is scientifically known by the name *Daucus carota*, is a widely consumed root vegetable that is rich in nutrients and a significant source of β carotene in addition to a significant number of vitamins and minerals that are frequently used for the production of juice (**Demir et al., 2001**). According to epidemiological research and other studies, they are high in beta carotene, ascorbic acid, and tocopherol, and are therefore regarded as vitamin enriched food. Recently, studies on people have shown that carotenoids also perform a protective effect for plants and animals against excessive sunshine (**Biesalski et al., 1996**).

Zinger (*Zingiber officinale*): *Zingiber officinale* (ginger) *Roscoe* is a plant that belongs to the Zingiberaceae family (**Singletary, 2010**). In addition to its many health benefits, ginger has been shown to have antioxidant, anti-inflammatory, immunomodulatory, and antibacterial properties by science. Its acceptability by consumers and growth in global manufacturing are fueled by these alleged advantages (**Rani et al., 2019**). Ginger is mostly used in cooking for its color, flavor, and preservation qualities. Ginger's bioactivity is mostly utilized in pharmaceutical and medical applications, along with its ability to suppress food pathogenic bacteria and oxidation (**Laelago Erseido et al., 2023**). Whenever food products are being processed for ingestion, ginger adds flavor and scent. The growing acceptance of this product among customers can be attributed to its tendency to enhance the flavor of prepared foods (**Jelled et al., 2015**). Various components of ginger, including shogaols, zingerones, and gingerols, have distinct therapeutic and nutritional properties (**Liu et al., 2017**). Ginger is incredibly flexible when it comes to enhancing the flavor of food and serving as medicine because it contains phytochemicals including phenylpropanoids, flavonoids, terpenes, and anthocyanins (**Sajilata et al., 2012**).

Probiotic fruits and vegetables juices

The nutrients found in fruit and vegetable juices differ from those found in the eatable parts of the fruits and vegetables, they include minerals, vitamins, and polyphenols from the fruits and vegetables (**Zheng et al., 2017**). Better immune function, a healthier digestive tract, and an enhanced nutritional profile are some of the benefits of the probiotic infused beverages. By incorporating probiotic fruit and vegetable juices into a balanced diet, people can optimize the health and well-being advantages of these beneficial microorganisms. Studies have been conducted on the emergence of different fruit and vegetable juices (**Lillo-Perez et al., 2021**).

It is crucial to promote alternative options for dairy based drinks, such as plant-based probiotic drinks. Making premium functional beverages requires a solid understanding of the variety of plant sugars that can ferment as well as the essential quality markers of fermented goods. To satisfy the growing demand from customers, it is challenging to maintain the high viability of probiotics in fruit-and-vegetable-based drinks. The introduction of non-dairy probiotic products into the market offers consumers more flavor options and encourages them to lead healthy lives (**El Soda et al., 1978**).

Using fermentation technology to create different probiotic fermented juices from fruit and veggies not only increases the nutrient content of fruits and vegetables but also organically combines the probiotics and their metabolic products with prebiotics to promote intestinal health and aid in the prevention and treat chronic illness (**Rahman et al., 2023**).

In place of fermented dairy products, other fruit and vegetable juices can be utilized to create fermented beverages either alone or in combination. Fermentation extends the expected lifespan of vegetables and fruit drinks while enhancing their functional and nutritional qualities, all of which have positive health impacts. In recent times, a multitude of studies has concentrated on the manufacturing of processed non dairy synbiotic drinks, using various fruits or vegetables, like blended carrot orange juices (**Valero Cases et al., 2020**).

2. MATERIALS AND METHODS

2.1 Material collection and blend juice preparation

The fruit and vegetables including apples, carrots and beetroot were purchased from the local market in Chinhut, Lucknow and stored at 4°C and utilized in the experiment as soon as feasible. The fruits and vegetables were thoroughly washed with tap water and then sterile water to remove dirt and foreign particles. The washed apple, carrot, and beetroot were blanched in boiling water for 5 minutes, and then transferred the blanched vegetables and fruit in cold water to stop overcooking. After blanching the fruit and vegetables were peeled and sliced into tiny pieces. Then the juice of apple, carrot and beetroot were extracted separately by juicer and filtered the extracted juices with the help of muslin cloth. The blend of apple, carrot and beetroot was prepared by mixing the juices in the proportion of 5:3:1 by volume, added with 5ml of ginger juice. Then the blend was transferred to a sterile glass bottle for microbial inoculation.

2.2 Strains and cultures

Probiotic supplements were bought from the online cite and were used to ferment the mixed juice by adding its nutritious properties. Probiotic capsules containing multiple strains of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus gasseri*, *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus paracasei*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Saccharomyces boulardii* were used to ferment the juice in this study. The strains were reactivated in the luke warm water.

2.3 Addition of probiotic culture in blend juice

In the apple, carrot and beetroot blend, probiotic multiple strain culture was added in the proportion of 0.2g/l. Five sterilized bottles were taken and each bottle contained 95ml of blended juice in the proportion of 5:3:1 with 5 ml of ginger juice. 20mg of blend strain was weighed from a probiotic capsule to inoculate 100 ml of blend juice. To reactivate it, the strain was transferred in 5 ml luke warm water and the mixture was stirred until the strain was dissolved and reactivated. Then 5 ml of reactivated strain was added to each bottle containing 95 ml of blended juice. Then the mixed juice blend with probiotic strain was mixed well and incubated at 37°C in the incubator for 24 h, 48 h and 72 h respectively. After 72 h of fermentation the remained bottles with sample were stored at 4°C in the refrigerator for 28 days.

2.4 Stability of fermented mixed probiotic juice blend at 4°C

Samples were stored at 4°C under the refrigerator for 3 weeks (21 days) after the fermentation of 72 h. During the storage period, the samples were removed and the viability of probiotic culture, analysis of pH, titratable acidity and total soluble solids in probiotic blended juice were conducted at weekly intervals of 0, 7, 14, and 21 days.

2.5 Physicochemical and Microbiological Analysis

Evaluation of fresh juice (control) and probiotic mixed juice, after the fermentation of 48 h was performed by physicochemical analysis through parameters such as pH, TSS (Total Soluble Solid), acidity, moisture content, ash content, total solids, fat content, protein content, carbohydrate content and energy, were determined for both samples.

As a control the apple, carrot and beetroot mixed juice blend was made without the probiotic strain addition.

pH, Total Soluble Solids (TSS), acidity and total viable count at the regular and weekly intervals were conducted only on probiotic ABC blend juice.

Cell viability in fermented probiotic mixed juice

In the fermented probiotic ABC mixed juice, the viability of the cells was determined by serial dilution and pour plate method and expressed as CFU/ml. Serial dilution was performed in sterile 0.1% peptone water. 1 ml of probioticated mixed juice sample was serially diluted in 9 ml of 0.1% peptone solution up to 10^{-7} dilutions. MRS agar medium was poured into the Petri plates and immediately 1 ml of dilution was pipetted out and transferred onto the center of Petri plates containing MRS agar. The plates were gently swirled to ensure that the sample was distributed throughout the MRS agar medium. The agar plates were allowed to solidify and incubated in incubator at 32°C for 48 hours. Plates which contained 20 to 250 colonies were counted and colonies number were recorded as colony forming units (CFU) per ml sample.

pH and Total Soluble Solid (TSS) measurement

The pH and TSS of probioticated mixed juice were measured at the regular intervals (0, 24, 48, 72 hrs) of fermentation and at 7, 14, 21 and 28 days to assess changes throughout the storage period.

pH was measured by a pH meter. 10 ml sample was transferred to a beaker. After calibrating the pH meter with the standard buffer solution the electrode of the pH meter was dipped into the sample for minute and reading was recorded accurately.

A refractometer was used to determine the TSS content in terms of °Brix. The refractometer was calibrated using distilled water. The prism surface was thoroughly cleaned and dried. Then few drops of mixed juice sample were placed onto the prism surface. In the direction of good light, readings were taken from the scale through the eyepiece of the refractometer.

Titratable acidity

Titrabale acidity was determined by titrating probiotic mixed juice with 0.1N NaOH solution.

- 10 ml sample was pipetted and transferred to the volumetric flask of 100 ml.
- Made up the volume with distilled water.
- The diluted sample from the volumetric flask was pipetted and transferred to a flask.
- 1, 2 drops of phenolphthalein indicator added to the sample.
- 0.1N NaOH solution was filled in the burette.
- While swirling the flask the solution was titrated against the fruit juice sample until pink colour appeared, which indicates the endpoint.
- The titratable acidity value was derived by applying the following formula.

$$\text{Titratable acidity \%} = \frac{\text{ml of NaOH used} \times 0.1\text{N NaOH} \times 0.064}{\text{The volume of sample used} \times 100}$$

Determination of Moisture content

Moisture content was determined by drying method in an oven.

- The weight of an empty clean and dried moisture dish was taken.
- Well-mixed juice sample was weighed in the tared empty dish.
- The dish containing the sample was placed in a hot air oven at 105°C for 4 h.
- The dish was taken out and allowed to be cooled at room temperature in a desiccator.
- The weight of the dish with dry residue was taken and moisture content was calculated as follows.

$$\text{Moisture content \%} = \frac{(M_1 + M_2) - M_3}{M_2} \times 100$$

Where,

M_1 = Weight of empty moisture dish

M_2 = Weight of sample

M_3 = Weight of moisture dish with dried sample

Determination of Ash content

- An empty dry silica crucible was weighed.
- The weight of well-mixed juice was taken and transferred to a pre-weighed empty crucible.
- Placed the crucible of the sample on the hot plate for charring.
- It was then kept in muffle furnace and ashed at 550°C for 5 h to get off white colour.
- The crucible with ash residue was cooled in a desiccator and weighed.
- The percentage of ash content was calculated by the following formula.

$$\text{Ash content \%} = \frac{\text{Dry weight} - \text{Empty weight}}{\text{Sample weight}} \times 100$$

Determination of Total Solids

- The weight of the clean and dry Petri dish was taken.
- The weight of well mixed sample of juice was weighed in a preweighed Petri dish.
- The Petri dish was kept in the water bath at 70°C.
- The Petri dish was then transferred to a hot air oven at 105°C for 1 h.
- The Petri dish was taken out and kept in a desiccator to cool down.
- Weight was taken and total solids content was calculated by applying the following formula.

$$\text{Total solids \%} = \frac{W_{TS} - W_P}{W_S} \times 100$$

Where,

W_{TS} = Weight of Petri dish with dried sample

W_P = Weight of empty Petri dish

W_S = Weight of sample

Estimation of Fat

- 2 grams of juice sample was taken.
- The sample was hydrolyzed by adding 2.5 ml of ethanol and 10 ml 8N HCl.
- Sample was kept in the water bath for 1 h at 80°C.
- Collected the residue in filter paper by filtering the sample.
- Maintained the pH of the residue sample to neutral with hot water.
- Kept the filter paper containing residue in hot air oven for half an hour.
- Put the filter paper into a thimble and placed it into the soxhlet apparatus.
- Poured 90-100 ml of petroleum ether in the preweighed flask or fat cup and attached to the apparatus.
- Perform the distillation with the solution.
- Extract liquid for 6 hrs at a condensation rate of 6-7 drops so that the solvent drips from the condenser to the sample.
- Retrieve the flask from the apparatus and put it in the hot air oven to evaporate the remaining ether.
- Kept the flask in a desiccator to cool the flask and take the weight of the flask with fat extract content.
- Calculate the fat content by the following formula.

$$\text{Fat \%} = \frac{W_D - W_{EF}}{W_S}$$

Where, W_D = Weight of flask with content; W_{EF} = Weight of empty flask; W_S = Weight of juice sample

Estimation of Protein

The nitrogen content was determined by the Kjeldahl method. The total protein was obtained by multiplying the nitrogen content by a conversion factor of 6.25.

A. Digestion

- 0.5g of sample was weighed and taken into a digestion tube.
- 0.5gm of cupric sulphate and 3gm of K_2SO_4 (potassium sulphate) were taken in the same tube.
- 10 ml of H_2SO_4 (sulphuric acid) was added to the tube containing all weighed samples and digested on the digester at $210^\circ C$ to $250^\circ C$.

B. Distillation

- After digestion, the sample was diluted with distilled water to prevent the solidification of the sample after cooling.
- The tube of diluted sample was placed in the distillation instrument for recovery of ammonia.
- The aliquot of the digested sample was distilled with NaOH (40%).
- From the digested sample ammonia was distilled out and collected in a receiving flask containing 4% boric acid with a drop of mixed indicator (Bromocrysol green and methyl red).

C. Titration

- After collecting the mixture in the flask indicator was added and titrated with 0.1 HCl until a pink colour appeared, which indicates the endpoint.
- The protein content in the mixed juice sample was calculated by using the following formula.

$$\text{Protein content \%} = \frac{14.01 \times 6.25 \times N \text{ of HCl} \times T.V. - \text{Blank T.V.} \times 100}{\text{Sample weight} \times 1000}$$

Determination of Total Carbohydrate

The percentage of the carbohydrate content in the mixed juice sample was determined by using difference method. It was calculated by adding the percentage value of moisture content, ash content, fat content, and protein and then subtracting it from 100.

$$\text{Carbohydrate content} = [100 - (\text{moisture} + \text{ash} + \text{fat} + \text{protein})]$$

Determination of Energy

The total energy was determined by adding the energy contribution from carbohydrates, protein and fat in kcal. Carbohydrate and protein provides 4 calories per gram and fat provides 9 calories per gram. The energy from carbohydrates and proteins was calculated by multiplying each by 4 and the energy from fat by 9. Calculation of total energy was done by using the following formula.

$$\text{Total Energy (kcal)} = (4 \times \text{Carbohydrate}) + (4 \times \text{Protein}) + (9 \times \text{Fat})$$

Determination of Mineral content in the mixed juice

Mineral content including Sodium (Na), Potassium (K) and Calcium (Ca) in the mixed juice sample was determined by Flame Photometer. Mineral content such as Iron (Fe) was determined by the AAS (Atomic Absorption Spectrophotometer).

- 5 ml juice sample was measured and transferred in a digestion tube.
- 5 ml of nitric acid (HNO_3) was added to the sample and predigested the sample for about 8 to 12 hours.
- After, predigestion 10 ml of diacid (HNO_3 and $HClO_4$) (Nitric acid and Perchloric acid) was added in a ratio of 4:1.
- The predigested sample was digested on a digester at the temperature of $210^\circ C$ to $250^\circ C$ until brown fumes turned to white fumes.

- After the digestion, the sample was allowed to cool at room temperature. The sample was filtered with Whatman 42 filter paper into a volumetric flask of 25 ml and made up the volume with Milli-Q water up to the mark.
- The sample was analysed for metals or essential minerals by using Flame Photometer and Fe by AAS.
- Standard was prepared. Stock solution of 100 ppm was prepared from the certified standard solution of 1000 mg/l and working solution.
- The mineral content was calculated by the given formula.

$$\text{Mineral content} = \frac{(M1 - M2) \times \text{Volume}}{\text{Sample weight}}$$

Where,

M1 = Concentration of sample ; M2 = Concentration of blank

Sensory Evaluation

Sensory evaluation of juice samples was conducted in the Regional Food Research and Analysis Centre, Lucknow to determine the acceptability of consumers. The evaluation of fresh apple, carrot and beetroot mixed juice and fermented mixed probiotic juice after 7 days and 14 days of storage was carried out by using 10-member panelists between the ages of 21 to 45. Samples were evaluated based on flavour, colour, aroma, texture, taste and overall acceptability by using a hedonic rating scale ranged from 9 (liked extremely) to 1 (disliked extremely). The fresh mixed juice and probiotic mixed juice samples were served in volume of 20 ml each to individual panelist in the clear glasses which were coded with three random digits. In order to prevent the transfer of sensory attributes from one sample to another juice sample, drinkable water was offered to rinse the mouth in between the assessments. After evaluating both the samples panelist rated the samples from 1 to 9 according to flavour, colour, aroma, texture, taste and overall acceptability.

3. RESULTS AND DISCUSSION

3.1 Change in pH, TSS, Titratable acidity and Cell viability during fermentation.

The results of analysis of pH, Total Soluble Solids (TSS), titratable acidity and total viable cell counts (cell viability) during the fermentation process of 3 days at every 24 hours of intervals in probioticated mixed juice of apple, carrot and beetroot are indicated in Table 1.

Table 1: Effect of fermentation over 72 h on pH, TSS, Titratable acidity and Cell viability.

Time (Hours)	pH	Total Soluble Solids (°B)	Titratable Acidity (%)	Total Viability of cell (CFU/ml)
0	5.02	10	0.03	10 ⁴ CFU/ml
24	4.58	8.7	0.13	10 ⁵ CFU/ml
48	3.95	6.4	0.21	10 ⁶ CFU/ml
72	3.67	6	0.23	10 ⁵ CFU/ml

3.1.1 pH Analysis

pH of the probiotic mixed juice sample during the fermentation of 24h, 48h, and 72h are shown in figure 1. While analyzing the pH it was observed that the pH of the probiotic mixed juice sample reduced with the increased time of fermentation. The pH of the mixed probiotic juice sample was 5.02 at the time of inoculation and it reduced to 4.58 after 24 h of fermentation. After 48 h of fermentation, the pH was 3.97 and slightly reduced to 3.57 after 72 h. The decrease in pH resulted from the due to organic acid production by the microorganisms added that were producing it by utilizing the sugar present in the mixed juice.

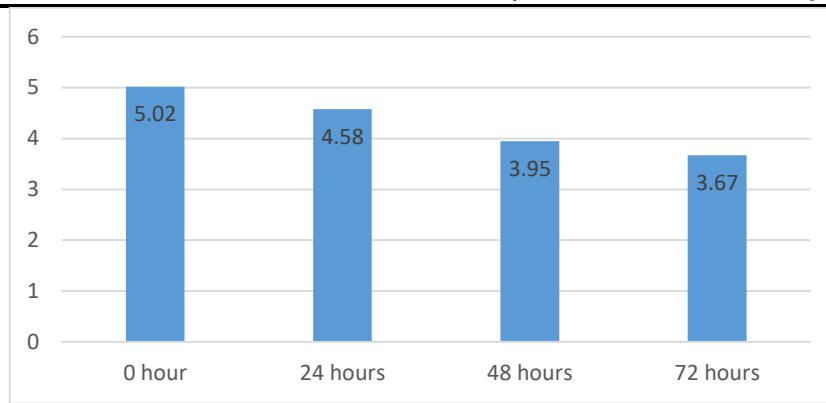


Figure 1: Changes in pH during the fermentation over 72 h.

3.1.2 TSS Analysis

The total Soluble Solids content of the probiotic mixed juice sample during the fermentation of 24h, 48h, and 72h are shown in figure 2. The TSS was determined using the refractometry method, it was observed that the TSS was decreased from 10° Brix to 6° Brix after 72 h. As shown in graph 2, the brix of probiotic drink during different times of fermentation, the brix of probioticated mixed juice is reduced. The reduced TSS value may be due to the effect of added microorganisms on the brix of mixed probioticated juice.

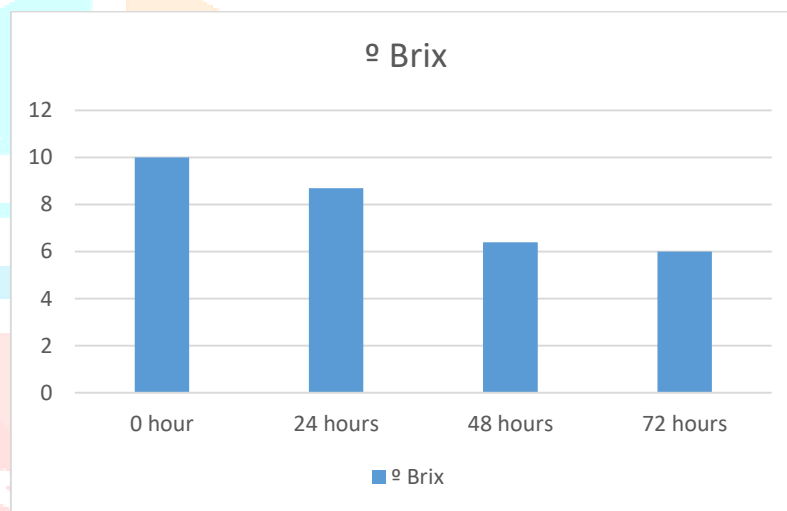


Figure 2: Changes in TSS (Brix) during the fermentation over 72 h.

3.1.3 Titratable Acidity

The titratable acidity in probioticated mixed juice sample were increased with the increase time of fermentation. In figure 3 it is shown that the acidity of probioticated mixed juice is increased to 0.23% after the fermentation of 72 h. At the time of inoculation the acidity was 0.03%.

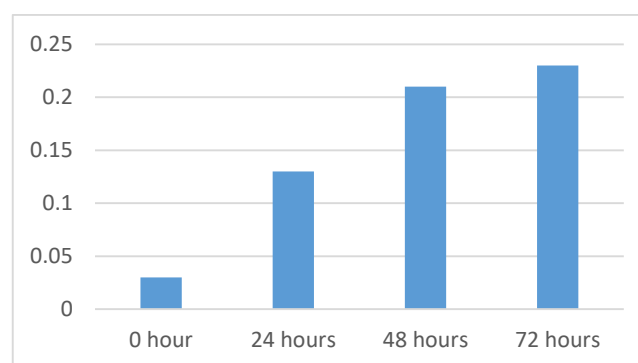


Figure 3: Changes in acidity in % during the fermentation over 72 h.

3.1.4 Total Viability of Cells

The growth of probiotics in mixed juice of apple, carrot and beetroot during the fermentation is shown in Figure 4. This result shows that the probiotic microorganisms were able to grow in the mixed juice. In the beginning, the count of probiotics at time 0 was 10^4 CFU/ml. After 24 h of fermentation in mixed juice, the probiotic count is 10^5 CFU/ml and after 48 h the count increased to 10^6 CFU/ml. Then after 72 h of fermentation the the count of probiotics in the mixed juice sample gradually starts to decrease. It is observed in some studies that according to scientists at the time of intake the probiotic count should be 10^6 CFU/ml to 10^7 CFU/ml for the healthful effect. So, the storage time was chosen after 48 h of fermentation of probioticated mixed juice.

3.2 Comparison of physicochemical parameters and proximate nutrients in fresh mixed juice without probiotic addition and probiotic mixed juice.

The physicochemical composition of fresh mixed juice without probiotic addition and fermented mixed probiotic juice after 48 h of fermentation are given in Table 2 and Table 3. The nutrients composition such as moisture, ash, total solids, fat, protein, carbohydrate and energy were determined and the results are given in the Table 3.

Table 2: Change in pH, TSS, acidity, viable count of fresh and fermented probiotic mixed juice.

Juice	pH	TSS	Acidity (%)	Viable count
Fresh mixed	5.02	10	0.03	10^4 CFU/ml
Fermented	3.95	6.4	0.23	10^6 CFU/ml

As shown in Table 2 the pH of fresh juice was 5.02 but after the fermentation process of probioticated mixed juice, the pH reduced to 3.95 after 48 h of fermentation. The reduction of pH in mixed juice was due to the production of acid by the probiotic microorganisms. The value of total soluble solids was also reduced. The TSS in fresh missed was 10^0 Brix and in probioticated mixed juice after the fermentation, it reduced to 6.4^0 Brix. The titratable acidity in fresh juice was 0.03% and in probiotic mixed juice, the titratable acidity increased to 0.23%. the viable count during the addition of probiotic after time 0 was 10^4 CFU/ml and the count increased to 10^6 CFU/ml in probioticated mixed juice sample of apple, carrot and beetroot.

Table 3: Proximate nutrients composition in fresh and fermented probiotic mixed juice.

Juice	Moisture (%)	Ash (%)	Total Solids (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Energy (%)
Fresh	87.95 %	0.40	12.04	0.46	0.06	11.13	48.9
Fermented	93.96 %	0.15	6.59	0.77	0.50	4.62	27.41

The nutrient composition such as moisture content, ash content, total solids content, fat content, protein content, carbohydrate, and energy content were determined. Some differences in fresh and probiotic juice were observed as shown in Table 3 The moisture content in fresh juice was 87.95%. After the fermentation of probioticated mixed juice, the moisture content in the juice increased to 93.96%. The ash content in fresh mixed juice without the addition of probiotics was 0.40 %. In probiotic mixed juice after the fermentation the the ash content was analysed as low as compared to the fresh mixed juice. The ash content in probiotic mixed juice was observed it reduced to 0.15 %. In fresh mixed juice, the total solids was 12.04 % and after determining the ash content in the probiotic mixed juice sample it was observed that it reduced to 6.59 %. In fresh mixed juice, the fat content was analysed and it was 0.46 % in the raw sample. The fat content in probiotic mixed juice was increased to 0.77 %. in comparison to fresh mixed juice the content of fat had no great difference.

In the fresh mixed juice sample the protein content was 0.06 % and after the fermentation of the probiotic mixed juice sample was when determined the protein content was increased to 0.50 % in the sample. In the result, this is due to the addition of probiotic microorganisms in the mixed juice sample. The probiotic microbes increase the protein content as compared to fresh juice without the probiotics.

The carbohydrate content in the fresh mixed juice and probiotic mixed juice was determined by their moisture content, ash content, fat, and protein content. The carbohydrate content in probiotic mixed juice was decreased in comparison to fresh mixed juice. In fresh mixed juice, the carbohydrate content was 11.13 % while in probiotic mixed juice carbohydrate content was 4.62 %.

The energy content in the fresh mixed juice and probiotic mixed juice was determined by adding the energy of carbohydrates, protein and fat. After the calculation by the given formula in methodology the energy was calculated and shown in Table 3. In fresh mixed juice, the energy was 48.9 % while in probiotic mixed juice, it decreased to 27.41 %.

3.3 Mineral content in mixed juice

The composition of minerals such as Fe, Na, K, and Ca of the mixed juice sample were determined and the calculated results are shown in Table 4.

Table 4: Mineral compositions of fresh mixed juice.

Minerals	Value (mg/100g)
Iron	0.434
Sodium	23.30
Potassium	40.42
Calcium	18.89

In the mixed juice, the iron was 4.34 mg/100g, sodium (Na) was 23.30 mg/100g, potassium (K) was 40.42, and calcium (Ca) was 18.89 mg/100g. The mineral content of individual fruits (apple) and vegetables (carrot and beetroot) was also determined. In apples, iron was 23.14 ± 1.10 mg/100g, sodium was 12.58 ± 1.01 mg/100g, potassium was 63.52 ± 0.47 and calcium was 72.41 ± 0.04 . In carrots, iron was 18.51 mg/100g, sodium was 73.58 mg/100g, potassium was 67.53 mg/100g and calcium was 83.09 mg/100g. In beetroots, iron was 1.30 mg/100g, sodium was 48.11 mg/100g, potassium was 96.24 mg/100g and calcium was 82.90 mg/100g.

3.4 Stability of probiotic mixed juice blend at the storage of 4°C after the fermentation of 48 h.

After the fermentation of 48 h the mixed juice was stored in the refrigerator at 4° C. Every 7th day of storage the stability of the probiotic mixed juice was determined. The pH, Total Soluble Solids (TSS), titratable acidity and total viable cell counts (cell viability) during the storage of 3 weeks at 4° C (7 days of intervals) in probioticated mixed juice of apple, carrot and beetroot are given in Table 5.

Table 5: Effect of storage at 4° C for 21 on pH, TSS, Titratable acidity, and Cell viability.

Day	pH	Total Soluble Solids (°B)	Titratable Acidity (%)	Total Viability of cell (CFU/ml)
0	3.95	6.4	0.21	10 ⁶ CFU/ml
7	3.92	6.4	0.21	10 ⁶ CFU/ml
14	3.91	6.5	0.20	10 ⁶ CFU/ml
21	4.01	6.9	0.08	10 ⁵ CFU/ml

3.4.1 pH analysis during the storage

pH of the probiotic mixed juice sample after storage of 7 days, 14 days, and 21 days are shown in figure 5. While analyzing the pH it was observed that the pH of the probiotic mixed juice sample was maintained at 3.9 after 14 days of storage and slightly increased to 4.01 after 21 days of storage. The increase in pH resulted from the due to breakdown of organic acid. In the figure and table, it is shown that pH is not much affected by the storage of upto 14 days.

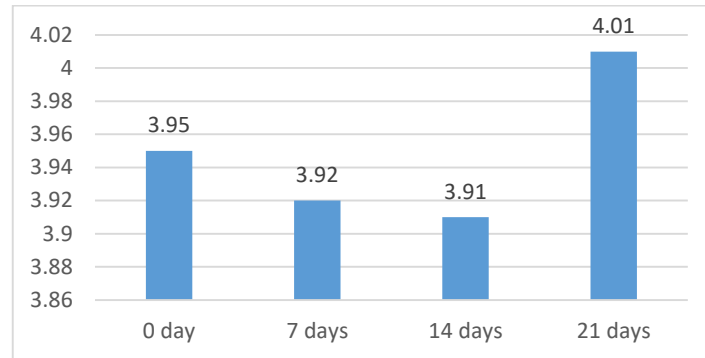


Figure 5: Effect of storage at 4° C on pH for 21 days of storage.

3.4.2 TSS Analysis during the storage

The total Soluble Solids content of the probiotic mixed juice sample during the storage time of 7 days, 14 days, and 21 days are shown in figure 6. The TSS was determined using the refractometry method, it was observed that the TSS was maintained from 6.4° Brix to 6.5° Brix and after the storage of 21 days TSS increased to 6.9° Brix. As shown in figure 6, the brix of probiotic mixed juice during different days of storage is maintained and increased with little TSS content it may be caused due to the breakdown of polysaccharides.

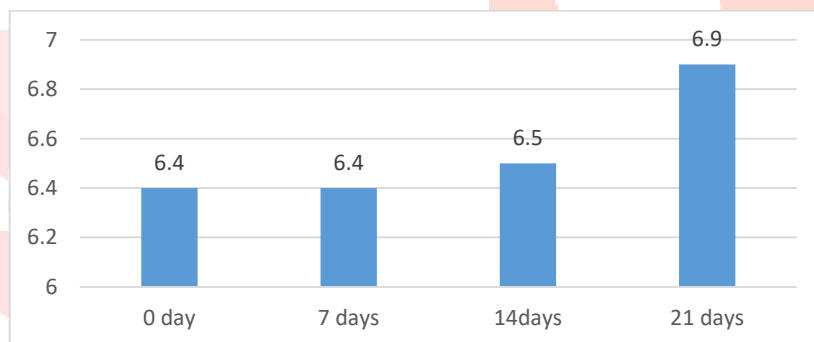


Figure 6: Effect of storage at 4° C on TSS in °Brix for 21 days of storage.

3.4.3 Titratable Acidity

The titratable acidity in probioticated mixed juice sample was decreased with the period of storage. In Figure7 it is shown that the acidity of probioticated mixed juice is reduced from 0.21 % to 0.08% after the storage of 21 days. The titratable acidity was maintained for up to 14 days of storage. The probiotic mixed juice sample was not much changed as shown in table 5.

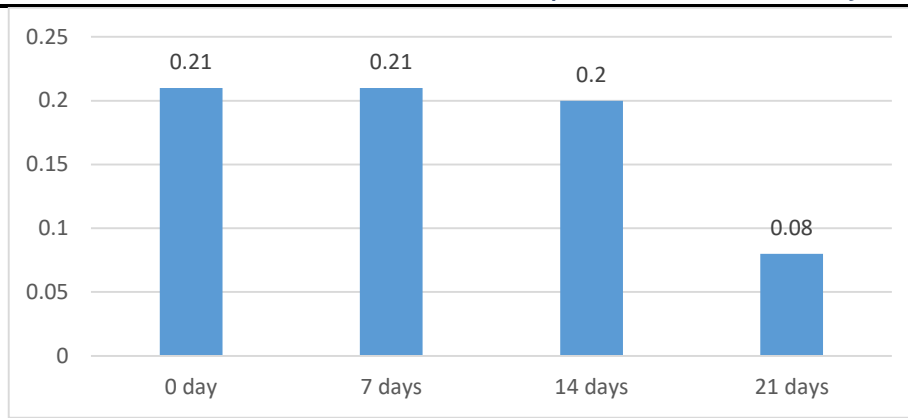


Figure 7: Effect of storage at 4° C on acidity in % for 21 days of storage.

3.4.4 Total Viability of Cells

The growth of probiotics in mixed juice of apple, carrot and beetroot during the storage days at 4° C is shown in graph 8. After the fermentation of 48 h, the viable count was 10^6 CFU/ml. In probiotic mixed juice till the storage of 14 days, there was no significant difference but after the storage of 21 days, the count was slightly decreased to 10^5 CFU/ml. It is observed in some studies that according to scientists at the time of intake the probiotic count should be 10^6 CFU/ml to 10^7 CFU/ml for the healthful effect. So, the consumption of the probiotic juice should be till the storage of 14 days.

3.5 Sensory Evaluation

Three mixed juice samples of different storage times was presented. The juices were chosen based on high cell viability after the fermentation and storage period. The score of sensory evaluation is given in table 6 and shown through figure 9.

Table 6: Sensory evaluation of fresh mixed juice and probiotic mixed juices of the 7 and 14 days of storage.

Samples	Flavour	Colour	Aroma	Texture	Taste	Overall acceptability
Fresh	8	7	8	8	8	7
7 day	7	8	6	7	6	6
14 day	7	8	5	7	5	6

The evaluation of fresh mixed juice without probiotic and probiotic mixed juice of the storage time of 7 days and 14 days was conducted. Fresh juice was more acceptable by consumers as compared to probiotic mixed juice. In the context of flavour, colour, aroma, texture, taste the fresh mixed juice was rated high in the score as compared to probiotic mixed juice. But the probiotic mixed juice is good in the health benefits. There was no significance difference in the storage time of 7 days and 14 days. The panelists only scored differences in the aroma and taste of mixed juice of the 7th and 14th days.

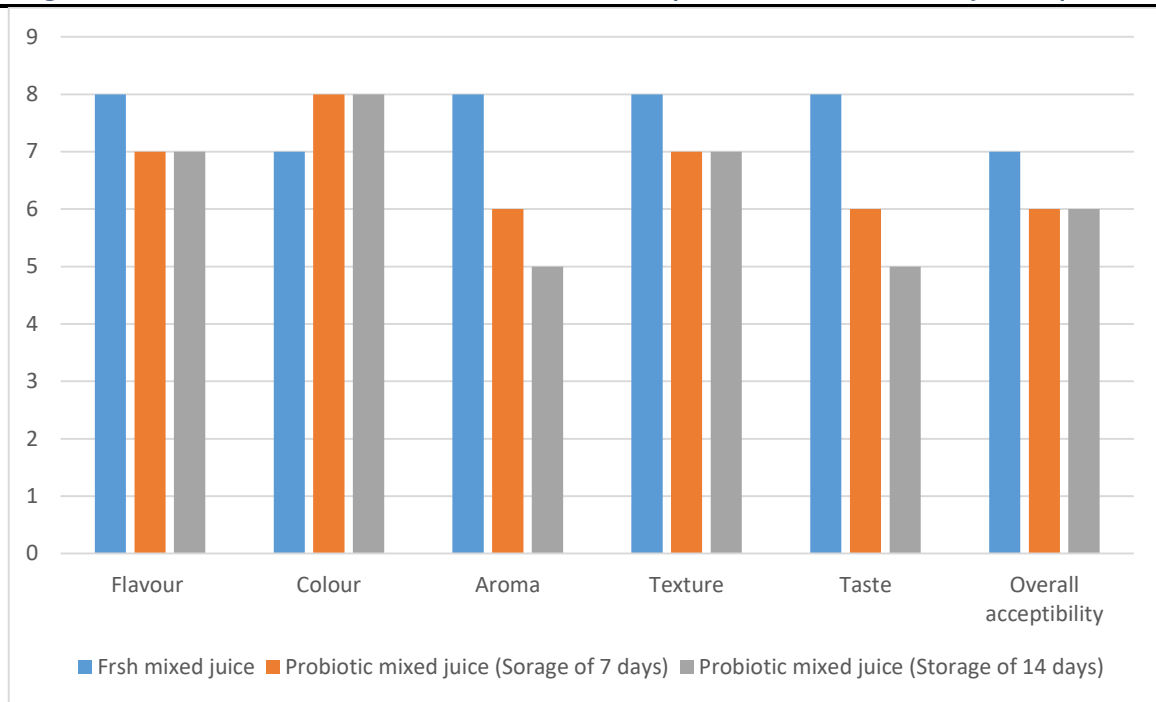


Figure 9: Sensory evaluation of fresh mixed juice and probiotic mixed juices of the 7 and 14 days of storage.

CONCLUSION

Based on the outcomes of this project, it is determined that mixed juice could be used as a source to make probiotic juice by fermentation. According to the study's outcomes, probiotic mixed juice of apple, carrot, and beet is produced and may even have health advantages. The juice showed beneficial changes in the pH level, total soluble solids (TSS), titratable acidity, and probiotic cell viability through fermentation. These changes show that probiotic microbes have transformed carbohydrates into organic acids, adding valuable chemicals to the juice.

Significant alterations in pH, Total Soluble Solids (TSS), titratable acidity, and cell viability were observed during the course of the 72-hour fermentation period. The juice's carbohydrates were being used by the added bacteria to produce organic acid, as evidenced by the pH progressively falling to 3.95 and 3.67, and it has a significant amount of beneficial bacteria (10^6 CFU/ml). Fruit juice's sugar and nutrient consumption can lead to an increase in probiotic bacteria during fermentation and reduce the (total soluble solids) brix levels. Acid production was indicated by an increase in titratable acidity. After 72 hours, cell viability started to decrease, emphasizing the need of selecting the best fermentation times to preserve healthy probiotic levels. So, 48 h of fermentation of probiotic juice was selected. Also, after fermentation, the probiotic mixed juice's nutrient composition improved, showing an increase in protein and a decrease in carbohydrate content. This implies a change to a product that is higher in nutrients, which may have further health benefits.

Fresh mixed juice and probiotic mixed juice after processing showed notable differences in proximate nutrients and physicochemical characteristics. The process of fermentation resulted in changes to the nutritional composition, it showed that pH reduced, increase in titratable acidity, and TSS levels reduced. The microbial activity resulted in a notable increase in protein content and a decrease in carbohydrate and calorie content. Different fruits and vegetables have different mineral compositions, which added to the mixed juice's overall nutritional profile.

Testing for stability at 4°C after fermentation revealed stable pH and TSS levels for up to 14 days, suggesting moderate stability. Titratable acidity dropped over time, most likely as a result of the decomposition of organic acids. For a period of 14 days, cell viability stayed comparatively constant, fulfilling the healthy probiotic criteria. Sensory evaluation however, shows that fresh juice was preferred over probiotic variants, emphasizing the significance of striking a balance between consumer appeal and health advantages.

In the conclusion of the study, probiotic mixed juice of apple, carrots, and beetroot is a convenient and healthy choice for people looking to add probiotics to their diet. Overall, probiotic mixed juice is a good choice for people looking for plant-based probiotic alternatives. To maintain the sufficient viability of probiotics and sensory qualities, it is advised that the best time to consume is within 14 days of fermentation.

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