The Development of Whey Based Probiotic Beverages with Amla and Its Quality Evaluation

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***Abstract*—Whey, a by- product of cheese and cottage cheese production, is a rich source of high-quality protein, essential amino acids, vitamins and bioactive peptides, making it as an excellent base for functional beverage. This study focuses on the development of a whey-based energy drink fortified with probiotic and amla juice to enhance its nutritional and functional properties.**

Amla is added as a natural Vitamin C source. Probiotics is incorporated to promote gut health, improve digestion and strengthen the immune system. Vitamin C, a powerful antioxidant was added to boost immunity, neutralize free radicals and enhance the drink stability by preventing oxidative degradation. The two different formulations were prepared with varying proportion of whey and amla juice to maintain probiotic viability and vitamin C stability during processing and storage. Sensory evaluation was conducted using a structured panel to assess attributes such as color, odor, flavor and taste. The physicochemical properties, including pH and acidity were analyzed to evaluate the stability of the product over time. The chemical composition of the beverage such as total phenolic content, protein, carbohydrate, ash, moisture, ascorbic acid and antioxidant were determined and the results were interpreted.

The developed whey-based energy drink demonstrated high sensory acceptance, prolonged shelf life and improved nutritional benefits, positioning it as a promising alternative to conventional energy drinks while catering to the growing demand for functional and health promoting beverages. We found that 50 %Whey and50% Amla juice was the best blend.

Index Terms*—Whey, Amla, Probiotics, Vitamin C, Energy drink and *Lactobacillus.

I. INTRODUCTION

Whey is one of the major by-products obtained during cheese and dairy processing and was previously considered a waste material. However, over the past few decades, scientific research has changed this perception and identified whey as a valuable functional ingredient with significant nutritional and health benefits (Walzem et al., 2002; Bardr et al., 2017). Whey contains a rich composition of proteins, lactose, minerals, vitamins, and bioactive compounds that make it useful in food and beverage applications. Due to its nutritional profile, whey is currently being utilized in sports drinks, therapeutic foods, and functional beverages (Marshall, 2004). The major proteins present in whey include beta-lactoglobulin, alpha-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, and glycomacropeptides. These components possess various biological properties such as immune support, antimicrobial activity, muscle development, and maintenance of overall health (Walzem et al., 2002; Arbizu et al., 2020).

Based on the method of production, whey is mainly categorized into sweet whey and acid whey. Sweet whey is obtained during cheese production through enzyme coagulation, while acid whey is produced during the manufacture of products such as yogurt and fresh cheese by acid coagulation (Zandona et al., 2021). Further processing of whey through filtration and drying techniques results in different forms such as whey protein concentrate (WPC), whey protein isolate (WPI), and whey protein hydrolysate (WPH)

(Nicolas et al., 2019; Mehra et al., 2021). These products are widely used in food industries because of their excellent nutritional and functional characteristics.

In recent years, consumer interest in healthy lifestyles and disease prevention has increased the demand for functional foods and beverages. Functional foods are foods that provide health benefits beyond basic nutrition and may contribute to improving physiological functions and reducing disease risk. Probiotics are one of the important components used in functional food development. They are defined as live microorganisms which, when consumed in sufficient amounts, provide beneficial effects to the host (Hill et al., 2014). Common probiotic organisms include species of Lactobacillus and Bifidobacterium, which are known for improving gut health and maintaining the balance of intestinal microflora (Mei et al., 2011).

The human gastrointestinal tract contains a large number of microorganisms that play an essential role in digestion, metabolism, and immune functions. Disturbance in the balance of these microorganisms can lead to various health problems. Probiotics help in maintaining a healthy intestinal environment by inhibiting harmful microorganisms and promoting the growth of beneficial bacteria. They can compete with pathogenic microorganisms for nutrients and attachment sites in the intestinal wall, thereby reducing infection risks (Chaucheyras et al., 2001). Probiotic organisms are also known to produce beneficial metabolites that improve digestion and support overall gut health (Makki et al., 2018).

Several studies have shown that probiotics provide additional health benefits beyond digestive functions. They may contribute to enhancing immune responses, reducing gastrointestinal disorders, improving nutrient absorption, and decreasing the risk of certain diseases (Martin et al., 2016). Research findings have also suggested their possible role in managing conditions such as obesity, diabetes, irritable bowel syndrome, and inflammatory disorders (Khani et al., 2012). Due to these beneficial effects, probiotics are increasingly incorporated into dairy products, beverages, and other food products (Borresen et al., 2012).

Amla (Phyllanthus emblica), commonly known as Indian gooseberry, is a highly valued fruit because of its nutritional and medicinal properties. It is considered one of the richest natural sources of vitamin C and contains various bioactive compounds such as polyphenols, flavonoids, minerals, and antioxidants. Traditionally, amla has been used in herbal medicine for treating several health conditions including digestive disorders, fever, common cold, and liver-related problems. Scientific studies have also reported antimicrobial, antioxidant, anti-inflammatory, and immune-supporting properties of amla (Ranjha et al., 2020).

The incorporation of amla into functional beverages has gained considerable attention due to its health-promoting potential and refreshing characteristics. The antioxidant compounds present in amla may help in reducing oxidative stress and improving overall health. Combining whey with amla can enhance the nutritional quality of beverages by providing proteins, vitamins, and bioactive compounds in a single product.

The development of probiotic whey beverages fortified with amla represents an innovative approach in the functional food sector. Such products may provide nutritional benefits along with probiotic effects, making them suitable for health-conscious consumers.

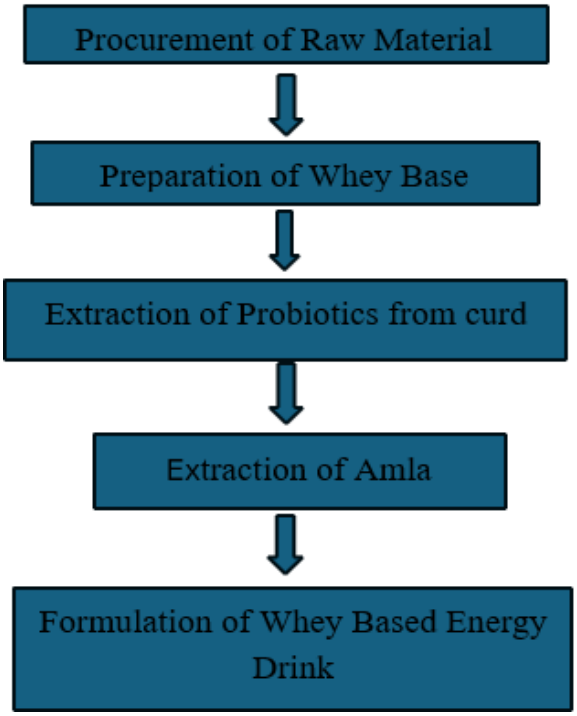
The growth and survival of probiotic organisms in whey-based beverages are important factors influencing product quality and effectiveness. Therefore, the present study focuses on the development of a whey-based beverage supplemented with amla juice and probiotics to evaluate its potential as a nutritious functional drink with improved health benefits.

II. MATERIAL AND METHODS

2.1. Sample and collection:

Curd sample was collected from home. Homogenized milk was collected from nearby dairy shop, and the whey was prepared in the lab by adding citric acid to the milk and the whey was strained. Amla sample was collected from nearby fruit shop, and the juice was extracted from the fruit and strained.

2.2. Flow Chart:



2.2.1. Procurement of Raw Material

Homogenised cow milk is brought from the local market. The milk is heated up to 90˚C. Lactic acid is added to the boiling milk (1g lactic acid for 100ml milk) and keep stirring the milk until it curdled. Then the milk is cooled at room temperature upto 40˚C. Then the whey protein and curdled casein protein are separated using a filter and muslin cloth.

The separated whey is collected in the bottle and stored.

2.2.2. Preparation of Whey Base

The collected whey was filtered through a muslin cloth to move residual solids and impurities. The whey was pasteurized at 72˚C for 15seconds to ensure microbiological safety while retaining its nutritional properties. After pasteurization, the whey was cooled to 37˚C to create a favourable environment for probiotic incorporation.

2.2.3. Extraction of Probiotics from Curd:

Fresh curd was stirred and diluted with sterile saline in a 1:1 ratio. The mixture was centrifuged at 3000 rpm for 10 minutes to separate the probiotic rich supernatant. The supernatant, containing viable probiotic cultures, was inoculated into the cooled whey based at a concentration of 10^6 – 10^8 CFU/ml.

2.2.4. Extraction of Amla:

Fresh amla was thoroughly washed, deseeded, and blended with a minimal amount of water to obtain a smooth pulp. The pulp was filtered to extract amla juice rich in natural vitamin C. The extracted juice was immediately added to the whey-probiotic mixture at a concentration of 50-100 mg/100 ml to meet the recommended dietary allowance (RDA).

2.2.5. Formulation of Whey Based Energy Drink:

The bottles were washed and sterilised. The Whey and amla juice were taken in the different ratio i.e., 50:50 and 25:75. Then the probiotics culture is introduced into the mixture of whey and amla juice. Then let it in the incubation chamber for 12 hrs and then it is stored in the refrigerator at 4˚C.

2.3. PROBIOTIC CHARACTERIZATION:

2.3.1. Growth at different Acid tolerance:

To check the growth of isolates at various pH, MRS broth supplemented with different pH 2, 3, 4, 5, 6 was prepared, 1% of fresh culture was inoculated and then incubated at 37ºC for 28 hours. During incubation, extent of growth was recorded objectively based on visible turbidity marked as double positive sign (++) for maximum growth, single positive sign (+) for normal growth and negative sign (-) for no growth.

2.3.2. Growth at different NaCl concentration:

Overnight grown cultures were inoculated at 1% in MRS broth tubes adjusted to various concentrations of NaCl viz. 1%, 2%, 4%, 5% and 6% along with their respective controls. The cultures were incubated at 37°C. After 24 hours of incubation, extent of growth was recorded objectively based on visible turbidity marked as double positive sign (++) for maximum growth, single positive sign (+) for normal growth and negative sign (-) for no growth.

2.3.3. Different Phenol Concentration:

For the determination of phenol tolerance, test tubes containing MRS broth were adjusted with different concentration (0.1 0.5%) of phenol.

After sterilization, each test tube was inoculated with 1% (v\v) fresh overnight culture of LABs and incubated at 37˚C for 24 h. After 24 h of incubation their growth was determined by 620 nm filter absorbance of cell concentration by spectrophotometer.

2.3.4. Antibiotic Sensitivity test:
Sensitivity of probiotics strains towards the antibiotics being tested by using Kirby- Bauer disc diffusion technique. Used antibiotics for testing: Amikacin (30mcg), Gentamycin (10mcg), Tetracycline (30mcg), Clindamycin (2mcg) and Nalidixic Acid (30mcg). For this purpose, MRS agar inoculated with LAB and disc were placed. After the incubation period 24hours/37°C and the inhibition zone were observed to determine the antibiotic resistance of isolates.

2.3.5. Bile salt Test:
The method described by (Mabrouk et al., 2007) was used to determine the tolerance of examined lactic acid bacteria to bile salts. MRS medium containing 0.1%, 0.2%, 0.3%, 0.4%, 0.5% (w/v) bile concentration was inoculated with overnight culture of lactic acid bacteria. Viable colonies were counted for 24 hours incubation time on MRS agar and growth was monitored at OD 610nm.

2.4. PROXIMATE ANALYSIS OF THE PRODUCT:
2.4.1. Estimation of Ash:
The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. 5 ml of the liquid samples was placed in a crucible and ignited in a muffle furnace at 550^{O}C for 6 hours. It was then cooled in desiccators and weighted at room temperature to get the weight of the ash.
Calculation:

$$\text{Ash (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.4.2. Estimation of Moisture:
The Petri-dish was washed thoroughly and placed in oven to dry. 5ml of the sample was then placed in a pre-weighed Petri dish and then placed in an oven to dry at 105^{O}C for five hours. The dish and dry sample were transferred to desiccators to cool at room temperature before, being weighed again. The experiments were repeated until constant weight was obtained.
Calculation:

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample Taken}} \times 100$$

2.4.3. Determination of total phenol content:
Total phenolic content was estimated by Folin Ciocalteu's method. 1 ml of plant extract was positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. It was allowed to incubate for 2 hours at room temperature. Intense blue colour was developed. After incubation, absorbance was measured at 750 nm spectrophotometer using UV-visible Jasco V- 630 instrument. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of polyherbal formulation were expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass.

2.4.4. Determination of protein content:
Weigh, to the nearest 1 mg, a mass of the test sample chosen according to the expected nitrogen content so that the test portion contains between 0.005 g and 0.2 g of nitrogen and, preferably more than 0.02 g.

2.4.5. Estimation of Carbohydrates:
1.0 g of sample was taken into boiling tubes and hydrolysed by keeping them in boiling water bath for 30minutes to 1 hr with 5ml of 2.5 N HCL. It was cooled to room temperature and neutralized with solid sodium carbonate until the effervescence ceases and made up the volume to 100ml and centrifuged.0.5ml and 1ml of the supernatant were collected and used for further analysis. The volume was made up to 1.0ml in all the tubes including the sample tubes by the addition of distilled water. 4ml of 0.2% Anthrone reagent was added to each test tube followed by heated in a boiling water bath for 10minutes.Test tubes were cooled at room temperature. Dark green colour had appeared on heating the samples. The optimal density (OD) value of the coloured solution was then measured through 630nm wavelength in a colorimeter against blank. Standard graph was drawn by plotting the concentration of Glucose on X axis and optical density on Y axis. From the graph the amount of carbohydrate present in the sample was calculated.

2.5. PHYSICOCHEMICAL ANALYSIS:
pH, Titrable acidity, was determined to the product while stored at 4°C at regular intervals (1,3,5,7,10) Days.

2.5.1. pH:
pH was determined by standard pH meter.

2.5.2. Titrable Activity:
10 ml probiotic drink was taken with pipetted in a 100ml beaker and added 4-5 drops of phenolphthalein indicator. Then titrate it against 0.1N NaOH till a persistent pink color was obtained.
Calculation:

$$\text{Acidity mg/l} = \frac{\text{Titrate value} \times \text{Normality of NaOH}}{\text{Sample Taken}} \times 1000$$

2.6. MICROBIOLOGICAL DETERMINATION:
The viable count of lactic acid bacteria was examined to evaluate the shelf life of Whey-Amla Probiotic beverage, which was stored under 4℃ for 10 days. Day regular intervals (1,3,5,7,10) tests were evaluated.

2.6.1. Viable plate count:
Label 9.9 ml tubes 10-1 to 10-6 respectively. Label agar plates 10-2, 10-4 and 10-6. Aseptically remove 1.0 ml of probiotic drink with a sterile pipette and transfer it to the 10-2 dilution tube. Vortex the 10-2 tube and transfer 1.0ml to the 10-4 tube. Again, vortex the10-4 dilution tube and transfer 1.0ml to the 10-6 tube. Using a new sterile pipette, aseptically transfer 0.1 ml from the 10-2 dilution tube to the plate labelled 10-2, transfer 0.1 ml from the 10-4 dilution tube to the plate labelled 10-4 and transfer 0.1 ml from the 10-6 dilution to the plate labelled 10-6. Spread the inoculums on the surface of the agar in each plate using an alcohol- dipped, metal spreader, dip the spreader into the alcohol jar and quickly take it through the flame and let the alcohol burn off after each spreading. Do not allow the spreader to get too hot. Never hold the spreader in the spreader in the flame for more than a second.

2.6.2. Antioxidant activity of probiotic fruit beverage:
1ml of extract/fractions (20-100µg/ml) was prepared in distilled water and mixed with 2.5ml of phosphate buffer and 2.5ml 1% potassium ferric cyanide 50˚C for 20min. 2.5ml of 10% trichloro acetic acid was then added to the mixture and centrifuged at 3000rpm for 10 min. 1ml of aliquot of supernatant was mixed with 2.5ml of distilled water and 0.5ml FeCl3 (10%) and absorbance was measured at 700nm. Increase in absorbance was interpreted as an increase of reducing activity.

III. RESULTS AND DISCUSSION

The present study was conducted to develop a whey-based probiotic beverage fortified with amla juice and to evaluate its nutritional, physicochemical, and storage characteristics. Different formulations of whey and amla juice were prepared and analyzed for total phenolic content, protein, carbohydrate, ash, moisture, and ascorbic acid content during refrigerated storage for 10 days.

3.1. Isolation Of Lactic Acid Bacteria (LAB):
Lactic Acid Bacteria (LAB) were isolated from Curd sample. *Lactobacillus sp*. was isolated with De Man Rogosa and Sharpe agar (MRS agar) at 37ºC under aerobic conditions for 48 hours. The isolated colonies were then screened for further identification using morphological characterization. The phenotypic identification is the first step in the selection of potential probiotic bacteria

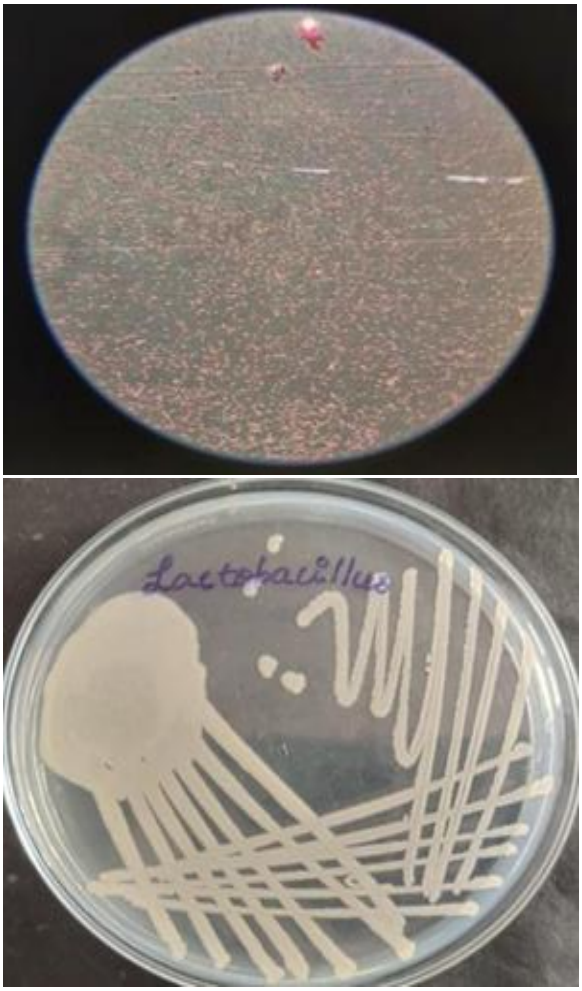


Fig 1: *Lactobacillus sp.*

3.2. Total Phenolic Content:

Phenolic compounds are important bioactive substances responsible for antioxidant activity. The total phenolic content of Product A and Product B was comparatively higher than Products C and D. On the first day of storage, Product B showed the highest phenolic content (61.62 mg GAE/100 g), followed by Product A (58.34 mg GAE/100 g). A slight reduction was observed during storage, reaching 59.65 mg GAE/100 g and 57.03 mg GAE/100 g respectively on the 10th day. This reduction may be due to oxidation and degradation of phenolic compounds during storage. However, the values remained relatively stable, indicating good retention of antioxidant compounds in whey-amla beverages.

Fig 2: Total Phenolic Content

3.3. Protein Content:

Protein is an important nutritional parameter contributed mainly by whey proteins. Product C showed the highest protein content (32.79 mg/100 g), followed by Product A (28.03 mg/100 g) and Product B (26.88 mg/100 g). Only a slight decrease in protein content was observed after 10 days of storage.

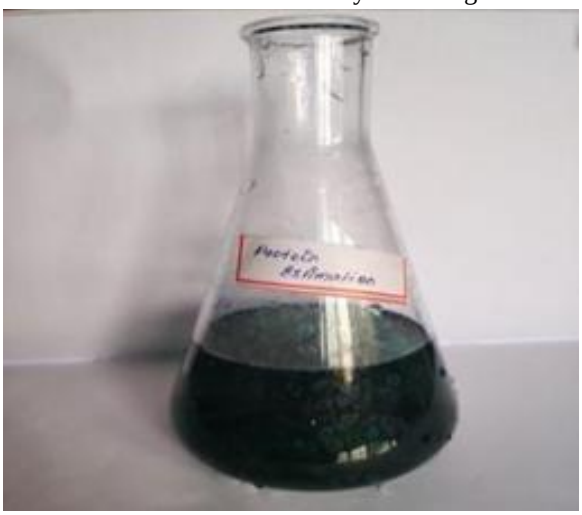


Fig 3: Protein Estimation

The reduction could be attributed to microbial activity and biochemical changes occurring during storage. Whey incorporation significantly improved the protein content of the beverage compared to amla-based formulations alone.

3.4. Carbohydrate Content:

Carbohydrate values ranged from 640–880 mg/ml on the first day and decreased slightly after storage. Product A exhibited the highest carbohydrate content (880 mg/ml), which reduced to 860 mg/ml on the 10th day. This reduction could be due to probiotic microorganisms utilizing carbohydrates as an energy source during fermentation and storage.

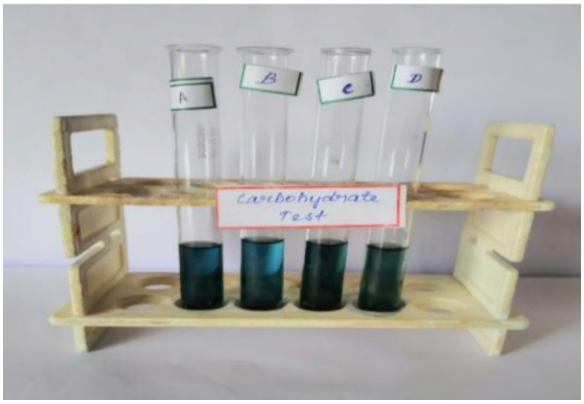


Fig 3: Carbohydrate Estimation

3.5. Ash Content:

Ash content represents the mineral composition of the beverage. Product A and Product B showed slightly increased ash values during storage, from 0.34% to 0.38% and 0.31% to 0.36%, respectively. The increase may be due to concentration effects caused by minor moisture loss during storage.

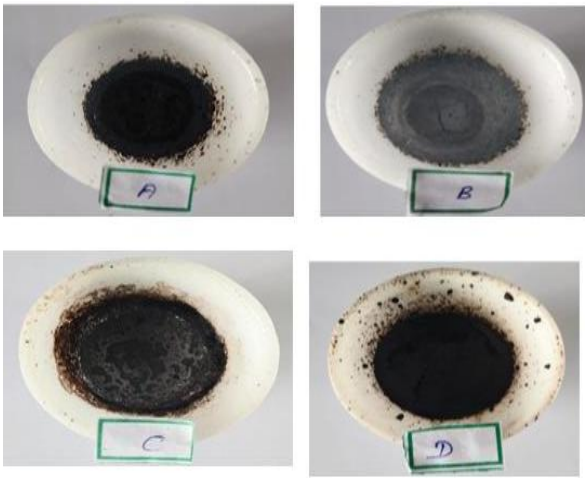


Fig 4: Ash Analysis

3.6. Moisture Content:

The moisture content of all formulations remained relatively stable during storage. Product D showed the highest moisture content (97.02%), whereas Product C showed comparatively lower values (90.96%). The minimal changes during storage indicate good product stability and packaging effectiveness.

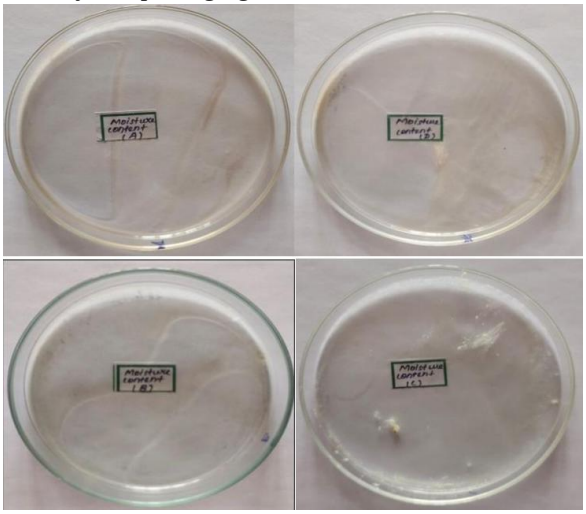


Fig 5: Moisture Content Estimation

3.7. Ascorbic Acid Content:

Ascorbic acid is one of the major nutritional components contributed by amla juice. Product D showed the highest ascorbic acid content (250 mg/100 g) on the first day, followed by Product B (208.42 mg/100 g). Slight reductions were observed during storage due to oxidation and temperature sensitivity of vitamin C. However, Product B retained comparatively higher levels of ascorbic acid throughout storage, indicating better stability.

Fig 6: Ascorbic Acid Estimation

3.8. Antioxidant Activity of Product (FRAB METHOD):

Indicate the fortification of Whey amla based probiotic beverage resulted in significant increase in absorbance value at increasing concentration on day 1 and day 10. Compared to day 1 and day 10 was increased, this was proportional to the level of their addition. The antioxidant properties were analysed using reducing power assay based on principles that substance which have reducing potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}) which then react with ferric chloride to form a ferric ferrous complex that has an absorption maximum at 610nm. The reducing power of product A, B, C and D tends to increase concentration.

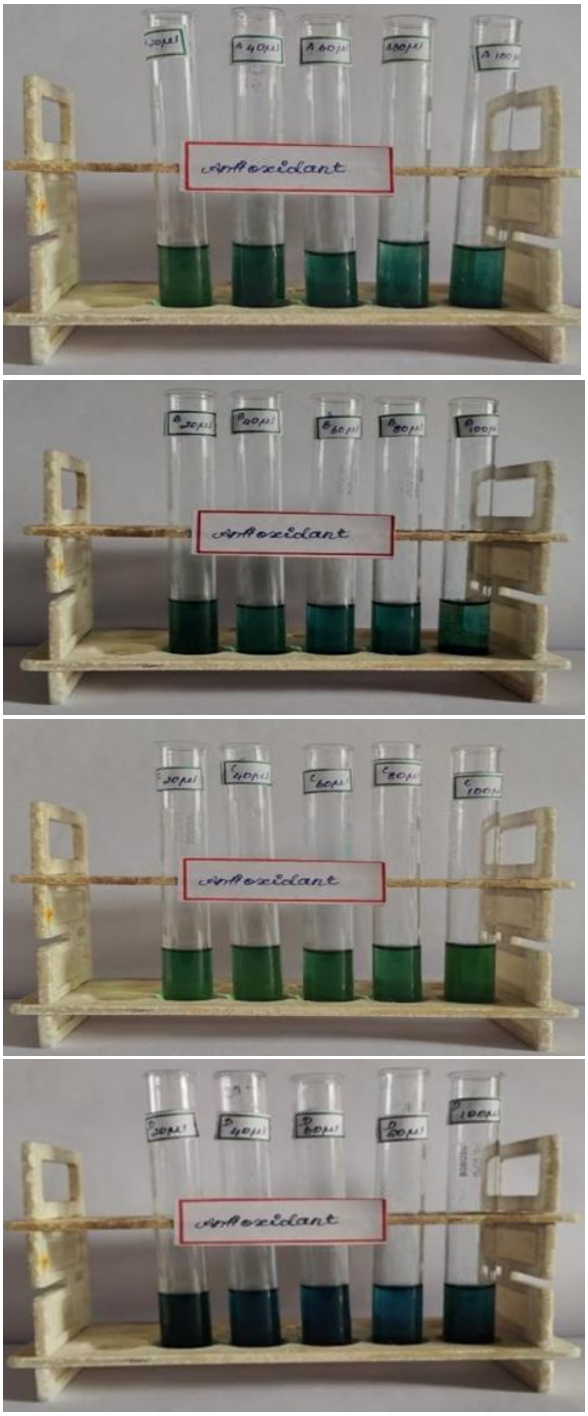


Fig 7: Antioxidant Activity

Table 1: Antioxidant Activity

S.NO	Storage Day	Concentration (µg/ml)	Absorbance OD Value			
			Product A	Product B	Product C	Product D
1	1st Day	20	0.68	0.97	0.27	0.97
		40	0.93	1.21	0.42	1.09
		60	1.01	1.42	0.55	1.21
		80	1.10	1.61	0.68	1.39
		100	1.28	1.79	0.79	1.45
2	10th Day	20	1.12	1.15	0.37	1.03
		40	1.28	1.29	0.52	1.26
		60	1.36	1.35	0.72	1.42
		80	1.49	1.48	0.84	1.64
		100	1.62	1.56	0.96	1.85

3.9. Effect of Storage on Product Quality:

Storage studies conducted under refrigerated conditions (4°C) for 10 days demonstrated only minor changes in nutritional parameters. The probiotic beverage maintained acceptable quality characteristics throughout storage. The slight reductions observed in phenolic compounds, protein, carbohydrates, and vitamin C may be associated with microbial metabolism and natural degradation reactions.

Table 2: Chemical Composition of the Product

S.NO	Storage Day	Test Parameter	Product A	Product B	Product C	Product D
1	1st Day	Total phenolic content (mg GAE/100g)	58.34	61.62	9.17	17.04
		Protein (mg/100g)	28.03	26.88	32.79	17.95
		Carbohydrate (mg/ml)	880	850	820	640
		Ash (%)	0.34	0.31	0.24	0.12
		Moisture (%)	93.44	93.96	90.96	97.02
		Ascorbic acid (mg/100g)	181.91	208.42	1.06	250.00
2	10th day	Total phenolic content (mg GAE/100g)	57.03	59.65	7.21	15.07
		Protein (mg/100g)	27.96	26.60	32.55	17.50
		Carbohydrate (mg/ml)	860	810	790	620
		Ash (%)	0.38	0.36	0.22	0.17
		Moisture (%)	93.06	94.17	91.10	96.99
		Ascorbic acid (mg/100g)	195.65	205.43	0.96	236.84

3.10. Overall Product Acceptability:

Among all formulations, the beverage containing 50% whey and 50% amla juice exhibited the best balance between nutritional value, antioxidant activity, sensory characteristics, and storage stability. The formulation showed improved protein content, higher antioxidant potential, acceptable acidity, and better probiotic viability. Therefore, the 50:50 whey-amla blend can be considered the optimum formulation for the development of a functional probiotic beverage.

Fig 8: Products and Control

IV. SUMMARY AND CONCLUSION:

The development of a whey-based probiotic beverage enriched with amla (Emblica officinalis) juice aims to utilize the nutritional richness of whey and the therapeutic properties of amla. The formulation incorporates *Lactobacillus sp.* as the probiotic culture, which are known for enhancing gut health and immune function. Amla juice, a potent source of Vitamin C and antioxidants, contributes to both the nutritional profile and sensory appeal of the beverage. The preparation process involves blending pasteurized whey with optimized concentrations of amla juice, followed by inoculation with probiotic strains and controlled fermentation. Key quality parameters evaluated include pH, titratable acidity, viable probiotic count, antioxidant activity, and sensory attributes such as taste, aroma, and overall acceptability.

Results typically show that the beverage maintains high probiotic viability throughout refrigerated storage (up to 10 days), with improved antioxidant activity due to amla integration. Sensory evaluation confirms good consumer acceptability, particularly at moderate amla concentrations (around 10%). Overall, the study confirms that whey-based probiotic beverages fortified with amla juice offer a functional, palatable, and sustainable option for health-conscious consumers while promoting effective whey utilization.

The current study revealed the feasibility of using whey for the development of probiotic Whey-Amla beverage. The results may give a great possibility to produce nutritional and functional amla beverage. With regards to, Sensory characteristics, Acidity, Total phenolic content, Protein, Carbohydrate, Ash, Moisture, Ascorbic acid, Antioxidant content, Probiotic cell Viability and Shelf life at 4˚C we found that 50 %Whey and50% Amla juice was the best blend. In addition, Whey-Amla beverage showed better Protein content and Ascorbic acid content than only probiotic amla juice. It may provide a Positive health effect among the Children, Athlete, Bodybuilder, Young adults and Health-conscious individual in developing countries. This study gives an importance knowledge to further work & development of probiotic whey-fruit based beverages regarding nutritional and functional properties important for and the food industry.

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