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Formulation and Evaluation of Lactobacillus spp enriched synbiotic grape drink for gut health promotion

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ABSTRACT :

The present study was aimed to develop and assess the efficacy of a novel synbiotic grape beverage enriched with Lactobacillus species. The beverage formulations were meticulously crafted by incorporating different concentrations of Lactobacillus species and subjected to fermentation to explore the potential benefits of probiotic and prebiotic synergy. Investigation into prebiotic fermentation dynamics elucidated the role of prebiotic substrates in promoting the growth and metabolic activity of probiotic bacteria, enhancing the synbiotic effects of the beverage. Physicochemical analysis revealed significant advancements in microbial viability, pH, acidity, sugar content, and antioxidant capacity following fermentation. Sensory evaluation indicated overall positive acceptance among panelists, with distinct flavor profiles observed in each variation. Furthermore, nutritional analysis showcased improvements in key parameters, including carbohydrate, protein, fat, and energy content, highlighting the potential of the beverage as a functional food product. In conclusion, the formulated synbiotic grape beverage presented a promising approach in promoting gut health and overall well-being, with implications for the functional beverage industry and consumer health consciousness.

Keywords: Synbiotic beverage, Lactobacillus, Grape drink, Fermentation, Physicochemical analysis, Sensory evaluation, Nutrient analysis

Introduction :

Nowadays the concept of using foods to promote a state of well-being, improve health, and reduce the risk of diseases has become the new frontier in the nutrition sciences and related fields. In this context, the development and contribution of functional foods must receive attention and should be key pillars of the healthcare system. Functional foods not only act as traditional nutrients, but they also have some additional beneficial effects such as improving health status, preventing and/or reducing nutrition-related diseases, and promoting a state of physical and mental well-being (Paul lachance, 2008).

In recent years, functional beverages have become increasingly popular due to their several specific health benefits. In the realm of beverage consumption, fruit beverages have emerged as popular choices, celebrated not only for their refreshing flavors but also for their inherent nutritional richness. Fruits, laden with essential vitamins, minerals, and antioxidants, offer a natural and convenient means to elevate the nutritional profile of beverages. Beyond mere refreshments, these drinks align with the global shift towards healthier alternatives, providing a welcome escape from sugary sodas (Gordon A ,2017).

Synbiotic beverages, combining the health benefits of probiotics with appealing flavors, contribute to the diversification of functional beverage offerings (Mintel, 2021). The term Synbiotics refers to the combination of probiotics and prebiotics that work together to promote gut health and overall well-being. Probiotics are defined by the World Health Organization as “live microorganisms that when administered in adequate amounts confer a health benefit on the host”. They help maintain a healthy balance of beneficial bacteria in the gut, improve digestion, boost the immune system, and may even have effects on mental health. On the other hand, FAO/WHO defines prebiotics as a non-viable food component that confers health benefits on the host associated with modulation of the microbiota. Prebiotics, nondigestible dietary compounds that selectively nourish beneficial bacteria in the gut, have emerged as key players in promoting gastrointestinal health and overall well-being (Gibson and Roberfroid, 1995).

When probiotics and prebiotics are combined they work synergistically to enhance each other's benefits. The importance of synbiotics lies in their ability to promote a balanced and diverse gut microbiota, which is essential for overall health. Synbiotics offer a convenient and effective way to support gut health and promote overall well-being. This cooperative strategy not only sustains the viability of probiotics but also amplifies their health-promoting effects, including improved digestion, strengthened immune function, and potential alleviation of gastrointestinal disorders (Marteau, 2003). The synbiotic beverage is formulated utilizing lactobacillus species isolated from sauerkraut as a probiotic source and grape juice as a prebiotic medium.

Lactobacillus species isolated from fermented cabbage, such as sauerkraut, have probiotic properties. They can colonize the gastrointestinal tract, where they confer health benefits by promoting gut health, enhancing digestion, and modulating the immune system [Marco et al., 2017].

Fermented cabbage, such as sauerkraut, undergoes biochemical transformations mediated by lactic acid bacteria, including Lactobacillus species. These transformations can increase the bioavailability of certain nutrients, such as vitamins and minerals, making them more accessible for absorption by the body [Di Cagno et al., 2013]. Also, certain components of grapes, such as soluble dietary fibers, can act as prebiotics.

The presence of natural sugars provides a quick energy boost, while the fibre content aids digestion and helps maintain a healthy weight. More over their low-calorie content and natural sweetness make them a nutritious choice for those looking to satisfy sweet cravings without compromising on health. Thus grapes, with their natural sweetness and unique flavor profile, serve as an excellent base for crafting synbiotic beverages.

Materials and methods :

2.1 Selection of Ingredients

Black grapes have been chosen for the preparation of this drink due to their exceptional attributes. Beyond their delightful flavor and rich color, black grapes are inherently prebiotic, fostering the growth of beneficial gut bacteria. Packed with antioxidants, vitamins, and minerals, they contribute to overall health. Their natural sweetness ensures a pleasant taste profile for the synbiotic drink.

Fresh ripened grapes with desirable sweetness and flavor procured from the local market were selected for the formulation of this drink.

2.2 Isolation and identification of lactobacillus bacteria

2.2.1 Preparation of sauerkraut

Homemade sauerkraut was used in the isolation of the bacteria. White cabbage in a mature state was used for its preparation. The obtained cabbage was cleaned of the core and outer leaves. Subsequently, the cabbage was chopped and mixed thoroughly with salt for about 15 minutes and added to the glass container. The closed container was incubated for fermentation at room temperature for about three days.

2.2.2 Tool Sterilization

All media and tools made of glass were washed and sterilized before use. The sterilization process of all mediums and tools was performed for 30 minutes followed by cooling for 15 minutes and then subjected to serial dilution.

2.2.3 Serial dilution and plating

Enumeration and isolation of LAB were performed according to ISO 15214:1998. To isolate the bacteria from the sauerkraut, serial dilutions were performed by adding the first 1 mL of sauerkraut to 9 mL of sterile distilled water (from 10−1 up to 10−7). Dilutions of 10−4,10−5 and 10−6 were used for spread plate inoculations. All the above-mentioned process was performed in a Laminar airflow chamber (LAF).

Following the serial dilution, plating technique was performed. 0.1 mL of the serial dilutions (10−4,10−5 and 10−6) were taken and spread onto the surface of de Man Rogosa Sharpe (MRS) agar media which is suitable for the growth of lactobacilli.

2.2.4 Incubation

After inoculation, the plates were incubated at 37°C for 24 hours. After incubation growth of Lactic acid bacteria was seen on the agar. After the successful growth of Lactic acid bacteria (LAB) on MRS agar, morphologically varying/distinct colonies were further isolated by streaking on new MRS agar plates using sterile-inoculating needles called loop and incubated at 37°C for 24-48 hours inside the incubator.

2.2.5 Identification of the bacteria

From each medium, a few colonies were randomly selected for identification. Well-isolated colonies were selected based on morphology (typical Lactobacillus morphology: small, round, creamy, and opaque). A sterile loop is used to streak selected colonies onto a fresh plate for purification. Incubate the streaked plate anaerobically, repeating the streaking process, if necessary, until pure colonies are obtained. The colonies are further subjected to an identification process.

The isolated LAB was subjected to phenotypic characterization. The phenotypic characterization of LAB was performed based on cell morphology, Gram reaction, and catalase activity.
The isolated bacteria were subjected to phenotypic characterization by viewing it in the microscope and rod-shaped elongated bacteria were seen. Then staining was performed followed by a catalase test and carbohydrate fermentation test. Gram staining was performed for all isolated colonies according

to the standard procedure. The appearance of a purple color after staining indicates that it is a gram-positive bacteria. For the catalase test, a drop of 3% hydrogen peroxide was added to a bacterial colony on a sterile glass slide and mixed well. The bacteria did not produce gas bubbles showing that the LAB is catalase-negative and the results are presented in Table 2.1.

Table 2.1

CRITERIA	RESULT
Growth at 15 degrees Celsius	+
Growth at 45 degrees Celsius	-
Staining	gram-positive
Catalase	catalase-negative

2.3 Formulation of grape drink

The selected optimum ripened, fresh grapes are thoroughly washed and made sure that they are free from any contaminants. Then the grape juice was extracted using a blender or juice extractor. Then the juice was strained and was heated to pasteurization temperatures (around 70-85°C or 158-185°F) for a short duration (15-30 seconds). Then the grape juice was allowed to cool (around 30-37°C) to a temperature suitable for inoculation with Lactobacillus bacteria.

The cooled grape juice was inoculated with the prepared Lactobacillus culture. Three experimental variations were designed to evaluate the impact of varying concentrations of Lactobacillus spp on the fermentation of grape juice. 1%, 2%, and 3% inoculation rates of Lactobacillus was incorporated in 100 ml of grape juice. Additionally, a control group consisting solely of grape juice without bacterial inoculation was included in the study.

The inoculated grape juice was allowed to undergo fermentation leaving some headspace for expansion. A controlled environment, preferably at 30-37°C (86-98.6°F) was maintained to support the Lactobacillus growth. The beverage was allowed to ferment overnight. The synbiotic grape beverages were bottled in sterile containers, leaving minimal headspace. Then the bottles were sealed securely to prevent contamination. Later the bottled beverage were refrigerated to slow down further fermentation and maintain probiotic viability.

2.4 Organoleptic evaluation

The formulated symbiotic grape drink was evaluated by a panel of 35 members. The beverage samples were randomly coded and presented to the panel members. Water was provided to rinse their mouth in between evaluations. The scorecard was distributed and the samples were evaluated based on a nine-hedonic scale and were rated for color, appearance, taste, flavor, consistency, and overall acceptability.

2.5 Nutrient analysis

The nutrient value of the formulated beverage was analyzed. The macronutrients like Energy, Fat, Protein, and Carbohydrates and micronutrients like vitamin C, beta carotene and folate were determined using standard procedures.

2.6 Microbial load analysis

The total bacterial count in the formulated product was determined by microbiological examination on the zeroth day and 15th day at regular intervals the growth of the bacteria was examined. The colonies formed were counted to determine the quality of food and to determine the shelf life of the product.

2.7 Antioxidant activity

The antioxidant content was determined by using the method called DPPH. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is a widely used assay for determining the total antioxidant capacity of compounds or extracts. In this method, the DPPH radical, a stable free radical with a purple color, reacts with antioxidants present in the sample, leading to a color change from purple to yellow. The degree of discoloration is directly proportional to the antioxidant concentration. The antioxidant activity was determined in terms of the ability of the antioxidants in the beverage to inhibit oxidation.

2.8 Statistical analysis

After the completion of the sensory evaluation by the panel members, the data was collected from the analyzed panelists and arranged for further statistical analysis. The data generated in the experiments such as sensory evaluation was consolidated and analyzed statistically as mean and standard deviation using SPSS software.

Results and Discussion :

3.1 Organoleptic evaluation

CRITERIA	Control	Variation I	Variation II	Variation III
APPEARANCE (Mean ± SD)	7.9143 ± 0.98	7.8286 ± 0.98	8.2000 ± 1.05	6.7429 ± 1.42
COLOR (Mean ± SD)	7.9714 ± 1.09	7.6571±1.30	8.0857±1.06	6.6571±1.69
FLAVOUR (Mean ± SD)	7.6857±1.64	7.4857±1.46	7.8571±1.14	5.9714±1.46
CONSISTENCY (Mean ± SD)	7.8571 ± 0.91	7.4000 ± 1.09	7.8857 ± 0.99	6.2857 ± 1.60
TASTE (Mean ± SD)	7.4571±1.73	7.3429 ± 1.30	7.9429 ± 1.21	5.2857 ± 1.52
OVERALLACCEPTABILITY (Mean ± SD)	7.3714 ± 1.57	7.3714 ± 1.13	7.7143 ± 1.04	5.9429 ± 1.37

From the above given table it is clear that the Variation II has obtained the highest score in all sensory parameters like Appearance, colour, flavour, consistency, taste and overall acceptability. This indicates that participants found this variation to be the most acceptable among all tested variations. The grape drink with Variation III received the lowest mean overall acceptability score of 5.9429. This suggests that participants found this variation to be less acceptable compared to the other tested variations.

3.2 Physicochemical analysis

Physicochemical parameters of the formulated drink

Parameter	Control	Variation II
pH	4.33	3.8
Titrable Acidity (%)	0.83%	1.2%
TSS(°Brix)	15°Brix	17°Brix
Reducing Sugar (%)	11.32%	9.68%
Total Sugar (%)	13.84%	11.01%
Moisture%	89.26%	89.68%
Ash %	0.30%	0.32%

Variation II (V2) exhibited a lower pH (3.8) compared to the control (pH 4.3), indicating increased acidity, potentially contributing to enhanced flavor and microbial stability. The higher titrable acidity (1.2%) in V2 compared to the control (0.83%) suggests a more acidic profile. Furthermore, Variation II demonstrated higher total soluble solids (TSS) at 17 brix, indicating increased sweetness and possibly improved sensory perception. The lower total sugar content in Variation II (11.01%) compared to the control (13.84%) further supports this trend, potentially influencing sweetness perception and consumer preference. Moisture content remained relatively consistent between the control (89.26%) and V2 (89.68%), indicating similar water retention properties and product stability. Ash content was marginally higher in Variation II (0.32%) compared to the control (0.30%).

3.3 Nutrient analysis

Nutrient content of the formulated grape beverage

CRITERIA	CONTROL	VARIATION II
Carbohydrate (g/100ml)	17.23g	15.17g
Protein (g/100ml)	0.42g	0.68g
Fat (g/100ml)	0.20g	0.22g
Energy (kcal/100ml)	66.2 Kcal	61.3 Kcal
Vitamin C (mg/100ml)	11.2 mg	18.26 mg
Calcium (mg/100ml)	15.68 mg	21.34 mg
Phosphorous (mg/100ml)	14.9 mg	23.04 mg
Magnesium (mg/100ml)	9.37 mg	13.32 mg

The nutritional composition analysis revealed notable differences between the control and Variation II formulations. Variation II (V2) exhibited a lower carbohydrate content (15.17g) compared to the control (17.23g). Conversely, Variation II demonstrated higher protein content (0.68g) compared to the control (0.42g), suggesting an increase in protein enrichment. This enhancement in protein content could potentially offer additional nutritional benefits, such as improved satiety and muscle support. Furthermore, both formulations showed marginal differences in fat. Interestingly, despite the differences in macronutrient composition, both formulations exhibited similar energy content, with V2 slightly lower at 61.3 kcal compared to 66.2 kcal in the control. Variation II exhibited a higher vitamin C content (18.26 mg) compared to the control (11.2 mg), suggesting an enrichment of this essential antioxidant vitamin. This enhancement in vitamin C content in Variation II may offer additional health benefits, such as improved immune function and antioxidant protection against oxidative stress. Variation II exhibited higher levels of calcium (21.34 mg) and phosphorus (23.04 mg) compared to the control (15.68 mg of calcium and 14.9 mg of phosphorus). The increase in calcium and phosphorus content could result from fermentation. Fermentation can lead to changes in pH levels due to the production of organic acids by microbial metabolism. These pH changes can affect the solubility and stability of minerals in the beverage, potentially leading to increased mineral levels as pH shifts favor mineral dissolution. Variation II also showed a slight increase in magnesium content (13.32 mg) compared to the control (9.37 mg). These findings underscore the importance of nutritional analysis in evaluating the composition and potential health implications of formulated beverages.

3.4 Microbial analysis

Microbial analysis of the formulated drink

Parameter	Storage period (days)	Control	Variation
Total Bacteriological count (CFU/ ml)	0th	2.56 × 10^4	1.95 × 10^4
	7th	4.67 × 10^4	2.14 × 10^4
	15th	5.00 × 10^4	2.87 × 10^4

The total bacterial count was found on the 0^{th} day, on the 7^{th} day, and on the 15^{th} day. The initial bacteriological count of the control group on the 0th day was 2.56 × 10^4 CFU/ml, which increased to 4.67× 10^4 CFU/ml on the 7th day and further elevated to 5.00 × 10^4 CFU/ml by the 15th day. In contrast, the initial bacteriological count of Variation II on the 0th day was slightly lower at 1.95 × 10^4 CFU/ml. However, the count remained relatively stable over the course of storage, with values of 2.14 CFU/ml on the 7th day and 2.87 CFU/ml on the 15th day.

The observed changes in bacteriological counts highlight the influence of fermentation and formulation adjustments on microbial growth and stability in grape juice. In the control group, the significant increase in bacteriological count over time suggests microbial proliferation. Conversely, Variation II exhibited a more favorable microbial profile, with a lower initial bacteriological count and relatively stable counts throughout storage. The presence of Lactobacillus spp in Variation II may have played a role in inhibiting the growth of spoilage microorganisms through competitive exclusion, production of organic acids, and other antimicrobial mechanisms.

3.5 Total anti oxidant activity

Antioxidant activity of the formulated beverage

Parameter	Control	Variation II
Total antioxidant value	54%	68%

From the above table, it is concluded that the antioxidant value was high in variation II (68%) when compared to the control (54%). The assessment of antioxidant activity in the formulated beverage revealed promising results, with significant differences observed between the control and Variation II (V2) formulations. Also increased level of vitamin C content from 11.2 mg in the control to 18.26 mg in Variation II highlights the effectiveness of the fermentation process and formulation adjustments in augmenting the beverage's nutritional profile. Vitamin C, a potent antioxidant, plays a crucial role in scavenging free radicals and protecting against oxidative stress.

The fermentation process, particularly with Lactobacillus spp, may have facilitated the release of antioxidant compounds from raw materials, enhanced bioavailability, and promoted the synthesis of novel antioxidant metabolites through microbial metabolism.

Conclusion :

In conclusion, the present study focused on the formulation and evaluation of a novel synbiotic grape beverage enriched with Lactobacillus spp. Through meticulous formulation variations incorporating different concentrations of Lactobacillus and subsequent fermentation, our research aimed to explore the potential benefits of probiotic and prebiotic synergy in enhancing the nutritional and functional properties of grape juice. The results of our study revealed significant advancements in nutritional, microbial and physicochemical attributes of the formulated synbiotic grape beverage. Fermentation with Lactobacillus bacteria led to a marked increase in nutritional composition leading to increased vitamin and mineral content. The drink also showed increased microbial viability, demonstrating the successful colonization and metabolic activity of the probiotic strain in the beverage matrix. Additionally, the fermentation process resulted in favorable alterations in pH, acidity, sugar content, and antioxidant capacity, indicative of enhanced nutritional quality and shelf-life stability. Moreover, sensory evaluation of the formulated beverage indicated overall positive acceptance among panelists, with variations exhibiting distinct flavor profiles and sensory characteristics.

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