International Journal of Recent Technology and Engineering (IJRTE)
ISSN: 2277-3878 (Online), Volume-14 Issue-3, September 2025

Formulation and Analysis of Dragon Fruit (Selenicereus Undatus) Fermented Water Kefir: A Non-Dairy Probiotic Alternative

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Abstract: ***This study examines the development, composition, and health benefits of probiotic drinks created by fermenting watermelon rinds with dragon fruit juice. The increasing demand for non-dairy, probiotic-rich drinks is being explored for Water Kefir, a fermented beverage recognised for its high lactic acid content, low alcohol content, and favourable microbial composition. Rich in antioxidants, vitamins, and minerals, dragon fruits are an ideal substrate that provides both flavour and nutritional value. This study evaluates the physicochemical, microbiological, and sensory properties of the resulting beverage. The results show that this drink contains important probiotic activities, supported by inexpensive microbial profiles, including Lactobacillus, Bifidobacterium, and yeast species. This study utilises water fermentation with dragon fruit juice, making kefir a sustainable and nutritious alternative to probiotics, suitable for lactose-intolerant and herbal consumers, and addresses the increasing preference for non-fine probiotic options.***

Keywords: Probiotic Beverage, Water Kefir, Dragon Fruit Juice, Lactic Acid, Antioxidants, Lactobacillus, Bifidobacterium, Nutrient-Dense, Lactose-Intolerant.

Abbreviations:
Total Soluble Solids (TSS)

I. INTRODUCTION

Individuals are becoming increasingly aware of the importance of healthy diets in enhancing their overall well-being. It is made from vegetables, grains, legumes, and fruits. Long-term non-milk beverage options are accessible worldwide, including the Bush Era, Haria, Maheu, Hardalie, and Kombucha. As a result, there is a growing demand for this traditional, probiotic, healthy drink. Water Kefir is an old-fashioned, handmade fermented drink with a fruity, acidic, and slightly foamy taste. It contains 2% lactic acid and has a minimum alcohol content (typically under 1%).

This can be achieved by following the water pitcher (starter) and fermenting the sweet water, which is added for its nutritional benefits and the sweet taste of dragon fruit juice [1]. The grain has a jelly-like texture and a yellowish–brown colour, appearing in various shapes and sizes, ranging from a few millimetres to several centimetres. When water is ingested, yeast (10^6 CFU/gram grain) and acetic acid bacteria (10^6 CFU/gram grain) can be present in a symbiotic relationship. Kefir offers a sustainable alternative to those interested in drinking fermented drinks during meals who do not consume animal products (vegan or herbal diets) or are sensitive or allergic to dairy products. When it is time to drink, the fermented kefir drink will be kept at 4°C [2].

The advantage of water kefir for health is that the microorganisms in the water are not pathogenic in the long term. In addition to the organic health benefits and the production of organic acids in fermented products, Shigella and Salmonella sp., like other harmful bacteria, may also be suppressed. In addition, the identified specific water keys exhibited probiotic properties. Probiotic potential bacteria were identified from fermented drinks and water kefir [3]. Dragon Fruit has a beautiful colour, smooth, mouth-sensitive pulp, and delicious black seeds embedded within it. It also has the advantage of being rich in nutrients. Dragon fruit belongs to the Cactaceae family and is in the genus *Hylocereus*.

Yellow-green or outer green perimeter segments, and the perimeter inner segments are white. The flowers can reach a length of up to 29 cm. It is a rectangular, rosy, red - colored fruit (length: 152, weight: 300 800 g) [4]. It consists of a wide, long-scale, red and green. The flesh is white, with small black seeds, and has a lovely texture and a delicious taste [5].

II. MATERIALS AND METHODS

A. Dragon Fruit and Its Health Benefits

100% pasteurised Dragon fruit juice is prepared. The initial pH of pasteurised dragon fruit juice is 6. Dragon fruits offer numerous health benefits and are used to help prevent various diseases. It supports eye health, enhances kidney

Manuscript received on 29 August 2025 | Revised Manuscript received on 12 September 2025 | Manuscript Accepted on 15 September 2025 | Manuscript published on 30 September 2025.
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and brain function, strengthens bones, and reduces the risk of high blood sugar, high cholesterol, and colon cancer. Its cholesterol–lowering effects are primarily due to the phytochemicals and plant sterols, which regulate cholesterol metabolism. Dragon fruit is also rich in bioactive compounds such as thiols, polyphenolics, antioxidants and betacyanin. Additionally, it aids digestion by neutralising harmful toxins, such as heavy metals, and helps the body build resistance against conditions like coughs and asthma [6].

B. Fermentation Dynamics of Water Kefir

The conventional production of water kefir is by soaking the kefir grains in a sugar solution of 6 – 30% with fresh or dried fruits for 2 to 4 days at room temperature, particularly at 21 to 25°C, with 6% to 20% (w/v) of kefir grains [7]. The second stage of fermentation can be performed by adding various fruit juices, such as apple, pineapple, lemon, and lime. After the grains are transferred, the mixture is left to stand for 24 hours to develop flavoured products [8]. The matrix's central component, glucan, is synthesised from sucrose during fermentation. During this stage, some microorganisms enter the water and ferment the sugar, while others remain attached to the grains [3]. This water kefir, obtained after fermentation, can be consumed immediately or stored at 4°C for 1 to 2 days, resulting in increased carbonation in the drink [8].

C. Fermentation Process

i. *Materials:* Medium 6-10% sucrose (Rapadura and cane sugar) and 6-30% water kefir grains, Rapadura Sugar, Salt- 2%.

ii. *Microorganisms*: Obtained from the water kefir grains. Airtight glass equipment under Anaerobic conditions.

iii. *Stages of Fermentation:*

- **First Stage Fermentation:** Inclusion of water kefir grains to potable water, under anaerobic conditions at 25℃ for 24 hours [9].

[Fig.1: 1st Stage Fermentation of Probiotic Drink]

- **Second Stage Fermentation**: Blend the probiotic juice with pasteurised dragon fruit juice under anaerobic conditions 25◦C and for 24 hours [9].

[Fig.2: 2nd Stage Fermentation of Probiotic Drink]

iv. *Equipment:* Incubator Sterile Glassware.

v. *Operational Procedure:* The flowchart for producing Tibicos beverages is illustrated in Fig. 3. In this process, Japanese water crystal types of grains are added to the water and sugar solution (which is already pasteurised and cooled) and incubated at a proper temperature, close to room temperature, at 25°C. As soon as the fermentation, the grains are segregated from the liquid medium by filtration through a sterile sieve. These grains are washed, dried, and kept in a cooling tank for the later inoculations. Although it could be profit-oriented, this flowchart applies to home-made and homespun water kefir. The fermented kefir drink is stored at 4°C and can be consumed readily. Suppose some acquaintance of the fermented beverage is added to the current fermentation along with the grains; in that case, the process is called "back-slopping". This practice is extensively used in various fermented foods [10]. Fermentation will occur between 20 and 37°C for 24 to 72 hrs., using 6% to 10% sucrose and 6% to 30% grains [9]. The most used source of sugar for fermentation is rapadura sugar [11], and the addition of pasteurised dragon fruit juice. However, due to the high requirements of microorganisms involved in water kefir grains, which can utilise various substrates, the beverage can be made from multiple sugar sources [12]. Fruit juice (300/500 ml flask) was inoculated with Water kefir grains and kept under anaerobic conditions at 25°C. Samples were taken at periodic intervals, and the colony-forming units were calculated. Additionally, the pH was measured. Rapadura sugar or organic blond sugar was added as a prebiotic to the probiotic juice, blended with pasteurised dragon fruit juice, and a check of the probiotic beverage was performed at 0 and 48 h of fermentation. Ten formulations

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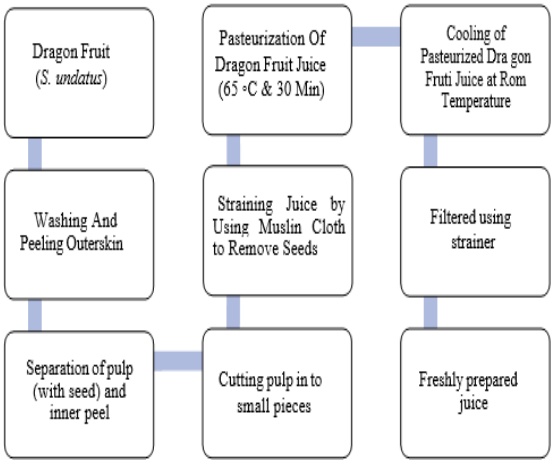
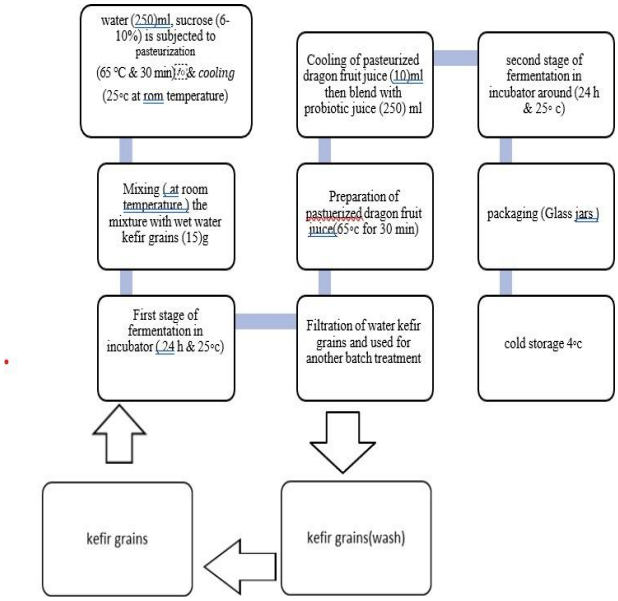
were created using the research surface methodology and then coded in alphabetical order.



[Fig.3: Development of Dragon Fruit Juice Flow Chart]



[Fig.4: Probiotic Juice Preparation with Pasteurised Dragon Fruit Juice]

vi. Physical Parameters

- **Total Soluble Solids (TSS):** TSS was measured using a refractometer based on the principle of light refraction, which correlates with the soluble solids. A few drops of the probiotic juice sample were placed on the refractometer prism, and readings were recorded at 25 ± 2 °C in °Brix. The prism was cleaned with carbonated water after each measurement. The °Brix scale primarily represents sucrose concentration, although samples may also contain other solutes, such as salts and proteins.
- **Turbidity:** Turbidity was measured using a turbidimeter, which compares the light scattered by the sample to that of a standard. The instrument was calibrated using standard turbidity solutions. Samples with low Turbidity (<40 NTU) were shaken and degassed before measurement. Readings were taken directly from the instrument scale (Association,1926).

vii. Chemical Parameters

- **pH:** The pH of the probiotic juice was measured using a calibrated digital pH meter (Hanna Instruments) at room temperature (25–30 °C). The meter was standardised with buffer solutions of pH 4.0 and 7.0. The electrode, rinsed with distilled water between uses, is immersed in the diluted sample (prepared by mixing 1 part of the sample with 1 part of distilled water) to record pH values accurately.
- **Carbohydrate Content:** Total carbohydrates were calculated by difference: % Carbohydrates = 100 – % Moisture – % Protein – % Fat – % Ash. This method includes reducing sugars such as sucrose, fructose, glucose, dextrins, maltose, and lactose, using the Anthrone method (IS 1656:2007).
- **Moisture Content:** Moisture was determined by oven-drying 5 g of the sample at 105 ± 1 °C for 5 hours, followed by cooling in a desiccator and weighing at 30-minute intervals until a constant weight was achieved (IS 1155:1968).
- **Ash Content:** Ash was measured by incinerating the dried sample in a muffle furnace at 550–600 °C until a grey residue was obtained, indicating total mineral content (IS 7874 Part 1:1975).
- **Fat Content:** Fat was estimated using the Mojonnier extraction method, which involves enzymatic digestion, solvent extraction with ethyl and petroleum ether, and gravimetric analysis after evaporation and drying of the solvent (IS 7874 Part 1:1975).
- **Protein Content:** Protein content was determined using the Kjeldahl method. Samples were digested in sulfuric acid with catalysts, followed by distillation and titration of released ammonia into a boric acid solution (IS 7219:1973).
- **Acid Insoluble Ash:** The ash residue was treated with 5N HCl, filtered, and the insoluble part was incinerated and weighed to determine non-digestible mineral content (IS 7874 Part 1:1975).
- **Crude Fibre:** Fat-free residue was boiled with diluted sulfuric acid and sodium hydroxide, filtered, dried, and incinerated to quantify indigestible plant material (IS 7874 Part 1:1975).
- **Energy Content:** The gross energy content was evaluated using bomb calorimetry, which measures the heat released upon complete combustion of the sample (IS 14433:2007).

- **Total Phenolic Content:** Phenolic content was measured using the Folin-Ciocalteu method with colourimetric detection at 650 nm after proper reagent incubation, with a standard curve constructed using B S A .

Table I: Microorganisms Present in Water Kefir Grains

Species	Reference
Bacteria	
Acetobacter fabarium	Gulitz & Associates (2011)
Acetobacter orientalis	The Gulitz Group (2011)
Acetobacter lovaniensis	Gamba and associates (2019)
Acetobacter indonesiensis	Gamba and associates (2019)
Acetobacter tropicalis	Gamba and associates (2019)
Bifidobacterium psychraerophilum	Laureys; Gulitz et al. (2011)
Bifidobacterium aquikefiri	De Vuyst and Laureys (2016)
Lactobacillus brevis	Pidoux (1989); Waldherr et al
Lactobacillus hilgardii	Pidoux (1989); Galli et al. (1995)
L.casei ssp. Casei	Pidoux (1989)
L.casei ssp. Rhamnosus	Gamba et al. (2019)
L. casei ssp. Pseudoplantarum	Galli & Associates, 1995
Lactococcus lactis ssp. Cremoris	Galli and associates (1995)
Lactococcus lactis ssp. Lactis	Pidoux (1989)
Leuconostoc citreum	Monar et al. (2014)
YEASTS	
Sacharomyces bayanus	Waldherr et al. (2010)
Sacharomyces cerevisiae	Franzetti et al. (1995)
Zygosacharomyces florentinus	Galli et al. (1995); Franzetti et al.
Zygosacharomyces fermentati	Gulitz and associates (2011)
Hanseniaspora valbyensis	Galli et al. (1995); Pidoux (1989);
Kloeckera apiculate	Galli et al. (1995); Pidoux (1989)
Candida valida	Pidoux (1989); Franzetti et al. (1998)
Pichia membranifaciens	Gamba and associates (2019)

viii. Microbiological Analysis

- **Total Plate Count:** The microbial load was assessed by performing pour plating of serial dilutions of the sample and incubating the plates aerobically at 30°C for 72 hours (approximately 3 days). Colony-forming units were counted and expressed as CFU/mL or CFU/g (IS 5402:2012, ISO 4833:2003).

ix. Sensory Analysis: Sensory analysis was done by using a 9-point hedonic scale. After drying, the samples were given to three different semi-trained panel members. Each panel member was asked to rate sensory attributes, including taste, colour, texture, and overall acceptability. ANOVA statistically analysed the results. Triplicates were made to experiment by taking the standard deviations.

Table II: Sample Code for Different Formulations

Ingredients (Sample)	Rapadura Sugar	Sugar	Water	Salt	Pasteurized Dragon Fruit Juice
A	3	15	200	0.6	20
B	3	15	250	0.6	15
C	3	15	250	0.6	15
D	4	20	250	0.6	15
E	5	20	250	0.6	10
F	4	16	250	0.6	10
G	4	16	300	0.6	10
H	2.5	10	250	0.6	10
I	1	4	200	0.6	10
J	**4**	**16**	**200**	**0.6**	**10**

Table III: Sample Proportion

Materials	Proportion
Water	200 (ml)
Rapadura	4 (g)
Sugar	16 (g)
Salt	0.6 (g)
Water kefir grains	5 (g)
Dragon fruit juice	10 (ml)

Table IV: Composition Medium

Casein's enzymatic breakdown	5,0 g
Extract via yeast	2,5 g
C6H12O6 glucose, anhydrous	1,0 g
Agar1)	9 g to 18 g
H_2o	1 000 ml

III. RESULTS AND DISCUSSIONS

A. Physical Analysis

Hylocereus undatus probiotic juice is estimated using a sample to estimate TSS and Turbidity.

- *Total Soluble Solids:* The TSS states the number of soluble solids in fruit; it affects the taste of the fruit because it can indicate the fruit's sweetness level.
- *Turbidity*: Turbidity is the measure of relative clarity of a liquid. The higher the intensity of scattered light, the higher the Turbidity.

Table V: Physical Parameters of Probiotic Drink

Sl. No.	Test Configurations	Outcomes	Units	Test Protocol
1.	Total Soluble Solids	4.6	(Brix)	FSSAI Part-A
2.	Turbidity	27	NTU	IS 3025(P10)- 1984(RA 2017)

B. Chemical Analysis

Hylocereus undatus probiotic juice is estimated by taking a sample and measuring its pH, Titratable Acidity, and Total Phenolic Content.

- *pH*: It is a measure of how acidic/basic water is. The range spans from 0 to 14, with seven being neutral.
- *Titratable Acidity*: An acidity measurement for wine and other foods is most helpful in determining the acid content for sensory description.
- *Total Phenolic Content*: The hydroxyl groups in plant extracts facilitate free radical scavenging. As a basis, phenolic content was measured using the Folin-Ciocalteu reagent in each extract.

Table VI: Chemical Parameters of Probiotic Drink

Sl. No.	Test Configurations	Outcomes	Units	Test Protocol
1.	pH	4.58	-	Hanna pH meter
2.	Titratable Acidity	1.89	%	Automatic Titrator
3.	Total Phenolic Content	3.2	Mg of analogous gallic acid (GAE)/g of sample	Folin- Ciocalteu's method (Singleton & Rossi method, 1965

C. Analytical Analysis

Hylocereus undatus probiotic juice was estimated using the sample to estimate Energy, Moisture, Carbohydrates, Proteins, Fats, Ash, Acid-insoluble Ash, and Crude Fibre.

- Energy is a quantitative property transferred to a body to perform work.
- Moisture is the presence of a liquid, especially water, often in trace amounts.
- Carbohydrates: These are sugar molecules, one of the primary nutrients in food.
- Proteins: It is a naturally occurring, extraordinarily complex substance that consists of amino acid residues joined by peptide bonds.
- Fats: Fats usually mean any esters of fatty acids or a mixture of such compounds.
- Ash: It indicates the inorganic residues present in food.
- Acid Insoluble Ash: The proportion of a sample not hydrolysed by 72% sulphuric acid.
- Crude fibre: It consists of cellulose and lignin plus mineral matter.

Table VII: Analytical Analysis of Probiotic Drink

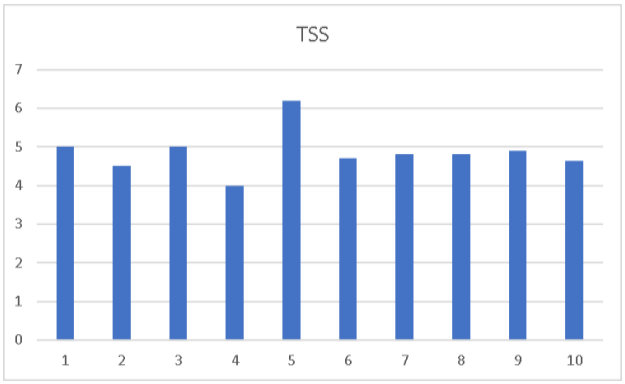
Sl. No.	Test Configurations	Outcomes	Units	Test Protocol
1.	Energy	11.36	kcal/100g	Clause 6.10.1 of IS 14433:2007
2.	Moisture	95	%	IS 1155-1968 Appendix A
3.	Carbohydrates	2.02	%	Annexe C of IS1656:2007
4.	Proteins	2.78	%	IS 7219-1973
5.	Fats	0.016	%	IS 7874(part 1)-1975
6.	Ash	0.18	%	IS 7874(part 1)-1975
7.	Acid Insoluble Ash	<0.01	%	IS 7874(part 1)-1975
8.	Crude fiber	<0.01	%	IS 7874(part 1)-1975

Table VIII: Microbial Analysis

Sl. No	Test Configurations	Outcomes	Units	Test Protocol
1.	Total Plate Count	1.5×10^{8}	CFU/ml	IS 5402:2012 (RA:2018) ISO 4833:2003

D. Sensory Analysis through Analysis of Variance

Three semi-trained panel members conducted a Sensory evaluation of Colour, Taste, Appearance, Odour, and overall acceptability. All fermentation experiments were performed in triplicate, and the results are expressed as the mean ± S.D. D (standard deviation).



Graph 1. TSS

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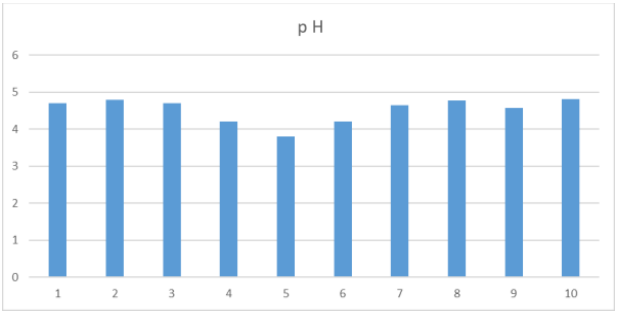
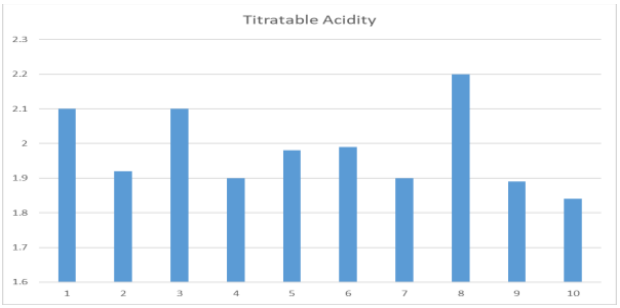
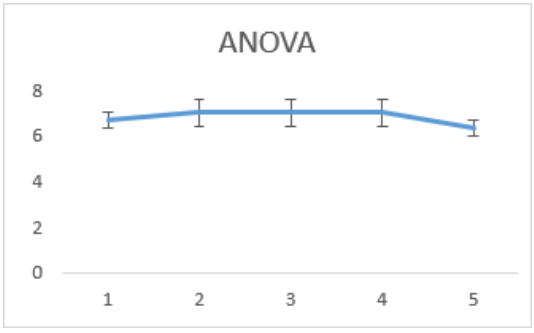


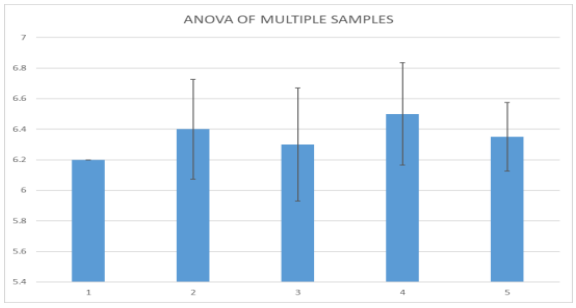
Graph 2. pH



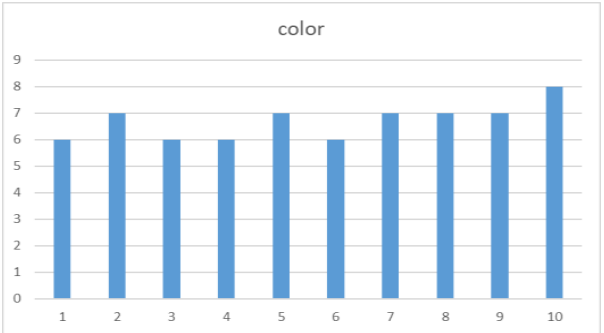
Graph 3. Titratable Acidity



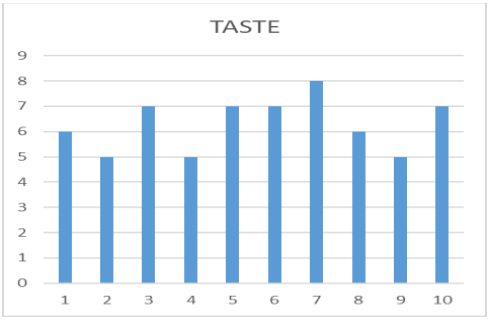
Graph 4. Sensory Analysis



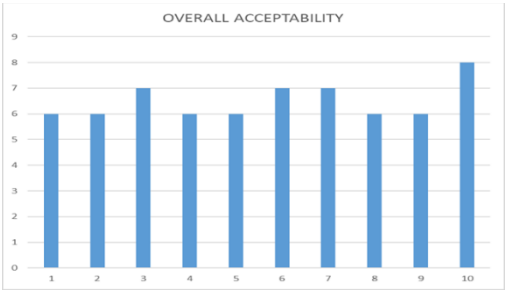
Graph 5. ANOVA of Multiple Samples



Graph 6. Color

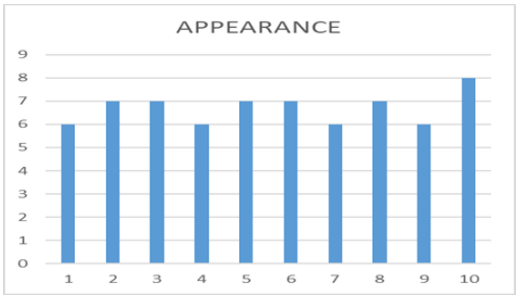


Graph 7. Taste

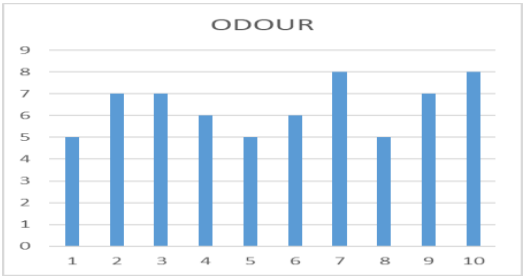


Graph 8. Overall Acceptability

IV. CONCLUSIONS



Graph 9. Appearance



Graph 10. Odor

Water Kefir is a traditional beverage made from the symbiotic fermentation of kefir grains with water. Water kefir is an artisanal and ancient fermented beverage, characterised by its fruity, acidic, sour, and slightly carbonated flavour, with a high lactic acid content and low alcohol content. Dragon fruit juice holds betalains, hydrocinnamates, flavonoids, proteins, and minerals. Water kefir grains consist of 70 microorganisms involved in a polysaccharide matrix (lactic acid bacteria sp., acetic acid bacteria sp., bifidobacteria sp., yeasts). This study aims to determine the feasibility of attributes and shelf-life treatments for probiotic dragon fruit juice. To study the physicochemical and

Retrieval Number: 100.1/ijrte.C829814030925
DOI: 10.35940/ijrte.C8298.14030925
Journal Website: www.ijrte.org

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microbiological parameters of the fruit. Samples are analysed for their physical parameters, chemical parameters, microbial analysis, and sensory (ANOVA) analysis. Dragon fruit is a suitable medium for probiotic bacteria, including Lactobacillus, Acetobacter, and Bifidobacterium strains. Consequently, it could be a different functional food pattern. The attributes of fermented beverages strongly depend on the range of genuine fermentable sugars, the probiotic strain(s), and nitrogen (protein).

The addition of prebiotic Rapadura sugar through the fermentation activity produced the production of lactic acid by lactobacillus sp., Acetobacter sp., and bifidobacteria., Yeast species moderately enhance the stability of fermented and probiotic cells. The part of the nitrogen source (both quality and quantity) must be evaluated to enhance the sensory taste of the dragon fruit juice beverage. Probiotic Lactobacillus sp., Acetobacter sp., Bifidobacterium strains, and other strains are suggested for producing a water kefir probiotic dragon fruit Beverage. The TSS and Turbidity were 4.64° Brix and 27 NTU, respectively. The obtained proximate analysis results were

Moisture – 95%, Proteins – 2.78%, Carbohydrates – 2.02%, Ash – 0.18%, Fats – 0.016%, Crude fibre and Acid insoluble ash were <0.01% respectively. The pH and acidity were obtained as 4.58 and 1.89%, respectively. The total plate count was analysed as 1.5 × 10^8 CFU/mL.

DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

- **Conflicts of Interest/ Competing Interests:** Based on my understanding, this article has no conflicts of interest.
- **Funding Support:** This article has not been funded by any organizations or agencies. This independence ensures that the research is conducted with objectivity and without any external influence.
- **Ethical Approval and Consent to Participate:** The content of this article does not necessitate ethical approval or consent to participate with supporting documentation.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Author's Contributions:** The authorship of this article is contributed equally to all participating individuals.

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