***Original Research Article***

**STANDARDIZATION AND QUALITY EVALUATION OF JAMUN FRUIT INCORPORATED VEGAN PEANUT KEFIR**

**ABSTRACT**

Kefir is a probiotic-rich fermented milk product prepared by fermenting milk with kefir grains, which are made up of numerous bacteria such as Lactobacillus kefiranofaciens and Lactobacillus kefiri, as well as yeast Saccharomyces cerevisiae living in symbiotic association inside the polysaccharide matrix. Consuming kefir provides a lot of health advantages. Peanuts can be considered as affordable low-cost ingredients to formulate plant-based milk alternatives as peanuts are affordable and nutrient-dense foods. This project aimed to create a vegan, plant-based kefir. Peanuts were bought from a local market in Chennai and then roasted, soaked, and blanched. After adding water in a 1:3 ratio, the blanched nuts were crushed in a domestic blender. The extract was cooked at 100°C for 10 minutes with continuous stirring after being filtered through a double-layered muslin cloth. Kefir culture was used to ferment the extract for 24 hours. The Jamun fruit, cardamom, and palm sugar were added to the kefir before being fully blended in a domestic blender to create the final fermented peanut milk kefir. Physiochemical, proximate, microbiological, and probiotic efficacy tests were performed on the product. The study's findings revealed that the protein content of the formulated product and the control cow's milk kefir was 3.23±0.04 g and 3.37±0.03 g, respectively. The prepared sample had an appreciable amount of calcium (45.2±0.3 mg). The final product was well-received and microbially safe for consumption. The probiotic count in Jamun fruit-incorporated vegan peanut kefir (JFIVPK) was estimated to be 25.9x106 CFU/ml. Hence the formulated JFIVPK can be considered a healthy choice for people with lactose intolerance, cow’s milk protein allergy, diabetes, Gastrointestinal infections, and hypercholesterolemia.

**Keywords**: Kefir, peanut, plant-based extract, Jamun fruit

**1 INTRODUCTION**

Kefir is an ancient fermented milk beverage that originated in the region of Caucasus and Tibet. Kefir is believed to arise from the Turkish phrase 'Keyif,' which signifies a pleasant feeling. Kefir is a classic and slightly effervescent beverage with an acidic flavor and a creamy texture that is made by fermenting milk with kefir grains. These kefir grains are formed by a complex community of lactose-fermenting and non-fermenting lactic acid, acetic acid bacteria, and yeast contained inside a protein and carbohydrate matrix **(Bengoa, Iraporda, Garrote, & Abraham, 2019; Rosa *et al.,* 2017)**. Lactobacillus kefiranofaciens, Lactobacillus kefirgranum, Lactobacillus paracasei, and Lactobacillus kefiri thrive in a symbiotic relationship with yeast such as Saccharomyces cerevisiae and Kluyveromyces lactis **(Sharifi *et al.,* 2017)**. These probiotics bestow health advantages on the host, mostly through the process of establishing or incorporating beneficial bacteria in the gastrointestinal system, which includes cholesterol-lowering and antioxidant effects **(Rasika *et al*., 2020; Bengoa, Iraporda, Garrote, & Abraham, 2019)**.

Plant-based extracts or non-dairy milk replacements are a rapidly growing segment of the functional and specialty beverage industry's innovative product development sector around the world. Lactose intolerance and hypercholesterolemia have become more common around the world, increasing the demand for non-dairy milk alternatives. Because of the aforementioned reasons, several plant-based milk alternatives have gained popularity in recent years. Peanut extract is one such plant-based milk substitute. The peanut (Arachis hypogaea) is an essential oilseed crop from the Fabaceae family. Black Jamun (Syzygium cumini L.) is a significant indigenous plant of the Myrtaceae family. Black Jamun is found predominantly in India's Gir forest region. The presence of anthocyanins is evidenced by the purplish-black color of the ripe Jamun. The pulp and seeds are used in traditional medicine **(Gajera *et al.,* 2017)**. The current study aimed to create Jamun fruit-infused vegan peanut kefir and investigate its nutritional and microbial properties.

**2 MATERIALS AND METHODS**

The raw materials used were Peanuts, Jamun fruit, Kefir culture, Cardamom powder, and palm sugar. Peanuts, palm sugar, Jamun fruit, Kefir culture, and cardamom powder were obtained from a local supermarket in Guindy. The project has been approved by the Independent Human Ethical Committee (IHEC) conducted by the Department of Home Science, SDNB Vaishnav College for Women, Chromepet, Chennai – 44, on 01/10/2021. The Protocol No - SDNBVC/HSE/IHEC/2021/23.

# 2.1 PREPARATION OF JAMUN FRUIT INCORPORATED PEANUT KEFIR

# The flow chart in Figure 1 depicts the preparation of JFIVPK. The peanuts are subjected to pre-processes such as roasting, soaking, and blanching before extraction of peanut milk substitute by grinding and filtering the peanut and water slurry in a 1:3 ratio. The JFIVPK was formulated as per the method suggested by Rosa *et al. (*2017) and Siddeeg *et al.* (2020) with slight modifications.

Addition of kefir grains to a glass jar

Addition of peanut extract

Seal airtight

Kept aside for 18 to 24 hours.

Addition of Jamun fruit, palm sugar, and cardamom

Grind the mixture

 Store in a glass jar at 4 degrees Celsius

*Figure 1: Preparation of JFIVPK*

**2.2 QUALITY ANALYSIS OF FORMULATED PEANUT KEFIR**

**2.2.1 PHYSIOCHEMICAL AND PROXIMATE ANALYSIS**

The physiochemical properties and proximate principles of the JFIVPK were analyzed, the properties include total titratable acidity, pH, viscosity, total soluble count, energy, protein, fat, moisture, ash, carbohydrates, iron, calcium, and magnesium. The techniques used for analyses are provided in Table 1.

*Table 1: Physiochemical and nutrient composition analysis Technique*

|  |  |  |
| --- | --- | --- |
| S.no | PARAMETERS | METHOD |
| 1 | Titratable acidity as Lactic acid | IS 1479 (Part 1): 1960 RA: 2012 |
| 2 | pH |
| 3 | Total Soluble Solids | FSSAI Manual 2016 - Fruits and Vegetables |
| 4 | Energy (By Calculation) | FAO Method |
| 5 | Protein (Nx6.25) | AOAC 20th Edition 986.25: 2016 |
| 6 | Total Fat | AOAC 20th Edition 954.02: 2016 |
| 7 | Total Ash | IS 1165: 2002 RA: 2013 |
| 8 | Moisture | AOAC 20th Edition-990.19: 2016 |
| 9 | Carbohydrates (By difference) | CTL/SOP/FOOD/262 – 2014 |
| 10 | Iron as Fe | AOAC 20th Edn.2016, 999.11 |
| 11 | Calcium as Ca | IS 5949 |
| 12 | Magnesium as Mg  |

**2.2.2 MICROBIAL ANALYSIS**

 The formulated JFIVPK was analyzed for its Total Bacterial Count (TBC) by plate count method, Yeast and Mould Count (YMC) by spread plate method, and Probiotic count.

**2.2.3 ISOLATION AND INVIVO PROBIOTIC EFFICACY EVALUATION**

The strain samples were diluted serially to 10-fold and then inoculated in the Man, Rogosa, and Sharpe (MRS) agar plates by the pour plate method. MRS agar plates were incubated at 37 °C for 48 h anaerobically. By streaking, morphologically differentiated colonies were determined and moved to fresh MRS agar plates. Finally, pure colonies were obtained after repeated subcultures and preserved for further study. The isolated probiotic strain was subjected to Bile salt tolerance, resistance to pH, Antibiotic sensitivity, phenol tolerance, and radical scavenging activity.

**2.3 STATISTICAL ANALYSIS**

The raw data obtained in this investigation was classified, coded, and analyzed using SPSS software (version 20.0 for windows, Chicago, IL, USA). Analysis of Variance (ANOVA) had been used to validate the research data, and the Ducan multiple range test was used to compare the means (DMRT).

**3 RESULTS AND DISCUSSIONS**

**3.1 PHYSIOCHEMICAL AND PROXIMATE ANALYSIS**

The physiochemical properties of peanut kefir such as total titratable acidity, Total solid count, viscosity, and pH were analyzed. The proximate parameters such as energy, protein, fat, ash, moisture, carbohydrates, iron, calcium, and magnesium were analyzed for the newly formulated JFIVPK and statistically compared with the control sample (cow’s milk kefir). The results are provided in Table 2.

*Table 2: Physiochemical and Nutrient composition of Peanut Kefir*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.no | Parameters | Control | JFIVPK | *p-Value* |
| 1 | Total Titratable acidity (%) | 0.71a±0.03 | 0.77a±0.04 | *0.144NS* |
| 2 | Total solid count (%) | 13.1b±0.35 | 9.1a±0.2 | *p<0.05* |
| 3 | Viscosity (cP) | 28.3b±2.0 | 8.2a±1.9 | *p<0.05* |
| 4 | pH | 4.25a±0.1 | 4.3a±0.04 | *0.301NS* |
| 5 | Energy (Kcal) | 88a±0.4  | 90b±0.6 | *p<0.05* |
| 6 | Protein (g) | 3.37b±0.03 | 3.23a±0.04 | *p<0.05* |
| 7 | Total Fat (g) | 3.83b±0.3 | 0.93a±0.04  | *p<0.05* |
| 8 | Total Ash (%) | 0.86b±0.02  | 0.71a±0.04  | *p<0.05* |
| 9 | Moisture (%) | 86.35a±0.37  | 90.4b±0.4  | *p<0.05* |
| 10 | Carbohydrates (g) | 4.47a±0.2  | 17b±0.4  | *p<0.05* |
| 11 | Iron (mg) | 0.27a±0.05  | 0.86b±0.03  | *p<0.05* |
| 12 | Calcium (mg) | 98.5b±0.5  | 45.2a±0.3  | *p<0.05* |
| 13 | Magnesium (mg) | 9.8b±0.2  | 5.39a±0.03  | *p<0.05* |

*Control - Cow’s milk kefir, JFIVPK - Jamun Fruit Incorporated Vegan Peanut Kefir*

*All values are the means of triplicate determination ± standard deviation*

*Significantly different (p*≤*0.05) by ANOVA. The same superscripts in the row indicate the same to each other and different superscripts in the row indicate different to each other are significantly different (p*≤*0.05) by DMRT (Duncan’s Multiple Range Test).*

The pH value of the JFIVPK sample was found to be 4.3±0.04 and the results were similar to the control sample. There was no statistically significant difference (*p>0.05*) identified between the control and the JFIVPK. The pH value of the JFIVPK sample was in correspondence to the pH value of Tiger nut yogurt (4.45±0.02) reported by **Ezeonu, Tatah, Nwokwu, & Jackson (2016).** The Total titratable acidity value of the JFIVPK sample was 0.74%. The acidity content of the JFIVPK sample was higher than the acidity value of cashew yogurt (0.55%) as observed by **Olayinka, Eugene, Olalekan, Richard, & Chuka (2018).** The Total soluble solids present in the JFIVPK sample account for 9.1±0.2% and were in line with the total soluble solids content of soy milk kefir was reported as 5.38% **(Egea, 2022)**. The viscosity of the JFIVPK sample was 8.2±1.9 centipoises. The present study’s result is in congruence with the viscosity of coconut milk kefir which was 7.36±1.2 centipoises by **Aussanasuwannakul, Puntaburt, & Treesuwan (2020)**. Generally, the plant-based extracts are low in fat, and the cow’s milk is high in fat thus there is a decrease in vegan kefir’s viscosity.

The energy, carbohydrates, and moisture content of JFIVPK were 90±0.6 Kcal, 17±0.4 g, and 90.4±0.4 %, respectively, and were higher than the control sample. There was a statistically significant difference *(p<0.05*) between the control sample and the sample for all the above-mentioned attributes. The energy value obtained in this study was lower when compared to the findings of **Lakshmi & Pramela (2018)**, who stated that the amount of energy present in Coconut milk Kefir was 117.5g. According to **Silva, Santos, Santana, Silva, & Conceicao (2018),** the carbohydrate content of the developed soy milk kefir was 8.5 ± 0.2g, which was lower when compared to the carbohydrate content of peanut kefir. The moisture level of food has a significant impact on its shelf life. The moisture content of the product was in conjugate with the moisture content of soymilk kefir which was found to be 87.57 ± 0.36% obtained by **Setyawardani & Sumarmono (2015)**. The JFIVPK contains 3.23±0.04g of protein, whereas cow’s milk kefir contains 3.37±0.03g of protein. The result of this study was supported by **Dadkhah, Pourahmad, Assadi, & Moghimi, (2011)**, where the protein present in soy milk kefir was 2.61±0.01g. The total fat and total ash content of JFIVPK were found to be 0.93±0.04 g and 0.71±0.04 % respectively. The fat value obtained from the current study was lower than the control sample and agreed with the results produced by **Silva, Santos, Santana, Silva, & Conceicao (2018),** who noted that the lipid content in soy milk kefir was 1.26 ± 0.03g. The amount of minerals included in the food product is represented by the amount of ash in the sample **(Ahsan *et al.*, 2015)**. The ash content was higher in peanut kefir when compared to black bean kefir (0.57 ± 0.02%) according to **Lim, Koh, Uthumporn, Maizura, & Rosli, (2019).** The variations could be attributed to the difference in the ingredients used.

 The calcium amount present in the cow’s milk kefir and JFIVPK obtained was found to be 98.5±0.5 mg and 45.2±0.3 mg respectively. The calcium content was higher in JFIVPK when compared with the calcium content of peanut-based kefir (33.47mg) developed by **Singh, Singh, & Dubey (2018)** and other plant-based extracts formulated by other researchers. The magnesium and iron content of JFIVPK was 5.39±0.03mg and 0.86±0.03 mg respectively. The magnesium content of JFIVPK is lower when compared with the results of **Shi, Kraft, & Guo (2020)** who concluded that almond yogurt contained (20.90±1.50 mg) of Magnesium. The iron obtained was slightly higher than the results reported by **Singh, Singh, & Dubey (2018)** for the peanut-based kefir where the iron content was found to be 0.06mg.

**3.2 MICROBIAL ANALYSIS**

 The microbial analysis of the JFIVPK sample was done as per the method suggested by **Thakur & Sharma (2017).** Total Bacterial Count (TBC) is the count of the number of bacterial colonies forming units present in the food sample, per gram. The total bacterial count and yeast colonies of JFIVPK were found to be 1.8x105 CFU/ml and 15.2x 105 CFU/ml respectively. According to the STANDARD FOR FERMENTED MILK, CXS 243-2003 the total bacterial count for Kefir can be a minimum of 107 CFU/ml and the yeast count can be a minimum of 104 CFU/ml. Yeast is a type of fungus that requires a warm, moist environment to produce food sources. The mold growth was absent in the sample which could be attributed to the clean and hygienic process followed during preprocessing. FAO[/WHO (2002)](https://www.sciencedirect.com/science/article/pii/S0022030214002549#bib0070) recommends a count of about (>107 CFU/mL) to have beneficial effects as a probiotic. The probiotics in JFIVPK were estimated to be 25.9x106 CFU/ml. Hence it can be concluded that the formulated peanut Kefir is a probiotic-rich beverage.

**3.3 ISOLATION AND INVIVO PROBIOTIC EFFICACY EVALUATION**

The Bile salt, pH, and Phenol tolerance of the isolated probiotic strain were analyzed and depicted in Table 3. The isolation and invivo probiotic efficacy evaluation was done as per the procedure suggested by **Anitha, Selvam, & Kulkarni (2018).**

*Table 3 - Probiotic efficacy of isolated strain*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sno | Incubation period | Bile salt Conc. 0.3% | pH | Phenol |
| pH2 | pH4 | 0.3% conc. | 0.5% conc |
| 1 | Initial | 150x104 CFU/ml | 150x104 CFU/m | 150x104 CFU/ml | - | - |
| 2 | 3 Hours | 145x104 CFU/ml | 143x104 CFU/ml | 148x104 CFU/ml | 22.04% | 13.16% |
| 3 | 6 hours | 141x104 CFU/ml | 67x104 CFU/ml | 146x104 CFU/ml | - | - |

 The isolated probiotic strain was tested for bile salt tolerance, pH tolerance, phenol tolerance, antibiotic resistance, and radical scavenging activity in in-vitro conditions to find its efficacy. These tests characterize the strain and check its survival rate in the human body. Bile salts are the focal elements of human intestinal fluid. Good bile salt resistance benefits the colonization of the isolated bacteria in the host GI tract **(Liu *et al.*, 2019)**. The initial growth of the identified probiotic strain was 150x104 CFU/ml. The growth rate of the strain decreased to 145x104 CFU/ml and 141x104 CFU/ml in the set intervals of 3 and 6 hours, respectively, with an increase in bile salt concentration to 0.3%. The strain has maintained probiotic development while surviving the extreme conditions, which is favorable to health.

 A significant criterion to be a good source of probiotics is its endurance to increase acid levels, present in the stomach. At pH-2 the initial count of the isolated probiotic strain was found to be 150x104 CFU/ml in the next 3 hours the growth rate of the strain decreased to 143x104 CFU/ml, further it is reduced to 67x104 CFU/ml in 6 hours intervals, whereas the strain in pH-4, the probiotic count was 148x104 CFU/ml and 146x104 CFU/ml at 3 hours and 6-hour time intervals respectively. The count of the isolated probiotic strain decreased in both pH-2 and 4 over some time, but the isolated strain at pH 4 was found to be well tolerant to the condition sustaining probiotic growth. Antibiotic overuse threatens their efficacy due to the promotion and spread of antibiotic-resistant bacteria **(Richardson, 2017)**. The probiotic strain isolated from JFIVPK was resistant to Oxacillin and Vancomycin, whereas it was sensitive toward Tetracycline, Chloramphenicol, and Amoxycillin respectively.

 Resistance to phenol is an important probiotic characteristic as phenol can be formed by the deamination of some aromatic amino acids by some bacteria and be able to exert a bacteriostatic effect **(Divya, Varsha, & Nampoothiri, 2012)**. The isolated probiotic strain growth was 22.04% at 0.3% concentration and reduced to 13.16% at 0.5% concentration. Hence the growth rate decreased with the increase in the concentration of phenol. The probiotic strain with the highest antioxidant activity has the greatest potential to scavenge DPPH radicals. The radical scavenging potential of the isolated strain from JFIVPK was found to be 53.3%. Henceforth it is evident that the isolated probiotic strain has good antioxidant properties.

**4 CONCLUSION**

According to the findings of the current investigation, the JFIVPK is a novel fermented product formulated with indigenous ingredients. The product's flavor and color profile have been tremendously improved by the addition of Jamun fruit pulp. The formulated product was determined to have a good probiotic count and efficacy. Therefore, the JFIVPK formulation can be regarded as a healthy option for the populace with lactose intolerance, cows milk protein allergy, and gastrointestinal infections. Furthermore, much scientific evidence supports the idea that plant-based extracts are a cheap source of protein and other micronutrients that can alleviate malnutrition and micronutrient deficiencies in developing nations when the availability of cow's milk supply is scarce and limited. But plant-based milk alternatives are deficient in one or more essential amino acids necessary for growth, nevertheless, JFIVPK possessed similar protein levels that were comparable to control protein. Furthermore, it can be concluded that the formulated vegan kefir is an inexpensive source of probiotics making it accessible to all sections of the population.

**ETHICS STATEMENT**

The research work was approved by the Institutional Human Ethics Committee (IHEC) of Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women (Autonomous), Chromepet, Chennai – 600 044, under the University of Madras. The IHEC approval number is SDNBVC/IHEC/24/01, dated 07/10/2024. All ethical guidelines were followed in the conduct of this study.

### ****INFORMED CONSENT STATEMENT****

 This study did not involve human participants, and therefore, informed consent was not required.

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