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Development and Analysis of Synbiotic Food Products using *Musa acuminata* Peels as a Strategy Towards Sustainable Utilization of Food Wastes

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Abstract

Food waste is a global issue, and improper waste management contributes to environmental and economic issues. This study aims to address this by repurposing nutrient-rich banana peels of *Musa acuminata* into sustainable synbiotic food. The peels were fermented with *Lactobacillus rhamnosus*, resulting in pickles with synbiotic effects. The developed pickles showed high organoleptic acceptability (8.67), with sufficient viable probiotics (5.3×10^9) and significant macronutrients, fiber, micronutrients, and phytochemicals. They also displayed free radical scavenging potential (88.09 %) and a desirable titratable acidity (7.68). The pickles were found to retain considerable viable *L. rhamnosus* after exposure to simulated gastrointestinal microenvironments. Furthermore, these were observed to be low-cost, manifesting a shelf life of 25 days (room temperature) with appreciable retention of sensory properties, acidity, and live probiotics, demonstrating their potential as a sustainable synbiotic food. This study highlights banana peels as a valuable resource in functional food innovation, promoting a zero-waste approach while imparting positive health effects.

Keywords: Banana peels; Functional; *Lactobacillus*; Pickles; Synbiotic; Waste.

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1. Introduction

Food waste, an ongoing global issue, constitutes a significant portion of the total waste [1]. This includes food that is discarded at various stages of the supply chain, from production and processing to distribution and consumption, which can culminate in loss of valuable resources and result in the emission of methane, a potent greenhouse gas contributing to climate change [2]. Wasted food is often edible but discarded due to cosmetic imperfections or lack of awareness regarding its scope of utilization. Fruit and vegetable wastes (FVW), accounting for 31.5 % of total production, comprise components including pomace, pulp, peels, and seeds that are abandoned during collection, handling, shipping, or processing phases [3]. Improper management of food waste, especially that generated from fruits, is a major environmental issue that contributes to pollution, resource loss, and public health risks [4]. Nonetheless, fruit byproducts have been known to harbor many essential

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nutritional and bioactive components, chiefly dietary fibers, vitamins, minerals, and antioxidants that are sometimes higher than the finished product. Hence, addressing food waste through valorization by repurposing them into high-value products may help in combating the mentioned menace along with promoting circular economy and food security [5,6].

Fruit peels, a predominant contributor to fruit waste, have been documented to be a reservoir of nutrients, phytochemicals, and prebiotics like inulin that may improve gut health and overall well-being if consumed through developed food or nutraceutical products. *Musa acuminata* (banana) peels, which make up 35 % of the fruit's weight, contribute significantly to food waste, with over 40 million tones discarded annually. Nevertheless, these being rich sources of dietary fibers, phenolic compounds, antioxidants, and essential minerals are valuable for developing food additives, dietary supplements, and functional foods [7,8]. As natural prebiotics, banana peels support the growth of beneficial gut bacteria, aiding in the production of short-chain fatty acids (SCFAs), which are crucial for promoting immune function and gut health. Moreover, their phytochemical content helps in preventing conditions like obesity, diabetes, and cardiovascular diseases [9]. Studies have proven synbiotics containing a careful combination of probiotics and prebiotics to manifest enhanced functionality versus their individual counterparts [10]. Accordingly, the current research focused on the utilization of waste materials obtained from fruit byproducts by the development of synbiotic functional food by combining the banana peels with optimum amounts of *Lactobacillus rhamnosus,* previously recognized as a suitable probiotic for synbiotic concoctions [11]. Utilizing fruit peels in synbiotic product development may not only promote a balanced gut microbiota but also align with sustainability by reducing food waste, fostering an eco-friendly food production system, and addressing health bestowed by the synergistic action of prebiotics and probiotics in the product.

2. Materials and Methods

2.1. *Sample and strain selection*

Banana (*Musa acuminata*) peels from the Pisang Awak subgroup, locally known as 'Kanthali' in West Bengal, India, were selected for their abundance as waste. *Lactobacillus rhamnosus* (ATCC 53103) was chosen for its proven gut health benefits and ability to withstand processing and gastrointestinal conditions, making it ideal for synbiotic product development.

2.2. *Product development and sensory evaluation*

Banana peel pickles were developed by fermenting banana peels in a probiotic brine solution (Table 1). Brine solutions were enriched with sugar and additional spices for taste enhancement. The best-accepted brine (B) was inoculated with probiotic *Lactobacillus*

rhamnosus. A sensory evaluation by 100 respondents (aged 18-25 years) using the 9-point hedonic scale helped identify the brine and probiotic variant (BP) for subsequent synbiotic product development [12]. BP was thereafter amalgamated with diverse proportions of banana peel blend and additional spices (BPV1, BPV2, and BPV3), fermented (36 h at 25 °C), and evaluated for sensory characteristics and probiotic growth. The prepared products were placed into clean, sterilized, and airtight jars. The formulation that received the greatest rating was further analyzed for probiotic, nutrient, functional, and physicochemical parameters versus the control (brine solution).

Table 1. Development of synbiotic product.

Product	Product Code	Water (mL)	Salt (g)	Sugar (g)	Pepper (g)	Chili flakes (g)	Ginger (g)	Probiotic (g)	Banana peel (g)	Garlic (g)	Cumin Powder (g)
Brine	B	100	2.1	1	0.15	0.25	0	0	0	0	0
Brine + Probiotic	BP	100	2.1	1	0.15	0.25	0	1.0	0	0	0
Brine + Probiotic + Banana peels (Fermented pickle)	BPV1	100	2.1	1	0.15	0.25	0	1.0	50	0	0
	BPV2	100	2.1	1	0.15	0.25	0	1.0	48	2	0
	BPV3	100	2.1	1	0.15	0.25	0	1.0	47	2	1

2.3. *Identification and culture viability*

The identity of probiotic *Lactobacillus rhamnosus* was confirmed through gram staining, catalase and oxidase tests, as well as lactic acid production. Probiotic growth in the best-selected variations, *viz.*, brine (B), brine with probiotics (BP), and banana peel pickle (BPV3), was analyzed using MRS broth incubated at 37 °C for 24 h. Growth was quantified using a colorimeter at 620 nm and colony-forming units (CFU) were calculated through serial dilution and plating on MRS agar [13].

2.4. *In vitro survivability in the human gastrointestinal (GI) tract*

Probiotic cultures from BP and BPV3 were tested for survivability in simulated gastric juice, bile solution, intestinal fluid, and varying pH and salt concentrations [14]. Gastric survivability was assessed using a pH 2.0 solution, while bile tolerance was measured in MRS broth with 4 % bile (Oxgall solution). Growth in the intestinal environment was evaluated with pancreatin solution at pH 7.5. The imitated gastric, bile, and intestinal solutions were prepared according to the composition recommended by Pal *et al*. [15]. Optical density (OD) readings at 620 nm were used to monitor *L. rhamnosus* viability.

2.5. *Nutritional analysis*

Nutritional and functional analyses were performed on the best variations of brine (B), brine with probiotic (BP), and banana peel product (BPV3), assessing various parameters

including macronutrients and micronutrients. The carbohydrate content was estimated using the anthrone method, while the protein content was determined using the Biuret method. Fat was extracted with petroleum ether (Soxhlet), and crude fiber was assessed through acid-base digestion [16]. Iron, magnesium, phosphorus, and calcium were quantified through a spectrophotometer (Hitachi, Japan) employing the ash prepared through muffle furnace incineration (500 °C) using the ferrozine, UV molybdate, calmagite, and OCPC methods, respectively, following the manufacturer's recommendation with respect to their individual standards. (Tulip Diagnostics, India). β-carotene was analyzed by acetone extraction, and vitamin C through titration with indophenol dye-based redox titration [17].

2.6. *Phytochemical analysis*

The samples were analyzed for their total phenol, alkaloid, flavonoid, and saponin contents. The Folin-Ciocalteau method was employed to determine the total phenolic content of the synbiotic pickle by measuring absorbance against a blank with reference to standard gallic acid at 765 nm. The flavonoid content was evaluated using the aluminum chloride method, which forms acid-stable complexes with the same. The intensity of these complexes was evaluated spectrophotometrically at 415 nm against quercetin through a calibration curve. The alkaloid content of a sample was determined by soaking samples in 10 % acetic acid in ethanol, macerating for 4 h, filtering, and concentrating. Ammonium hydroxide was added to a solution to form a precipitate, which was then washed and dried. Alkaloid content was calculated by estimating the weight of the precipitate with respect to the weight of the original sample. The saponin content was measured by combining sample extract with a reagent mixture (glacial acetic acid and sulfuric acid in a 1:1 v/v ratio), heating, and measuring absorbance at 527 nm against standard oleanolic acid [18].

2.7. *Physicochemical analysis*

The moisture content was determined using the thermogravimetric method by measuring the decrease in weight upon oven drying at 105 °C. The total soluble solids (TSS) were calculated by a refractometer. Titratable acidity (TA) was assessed by titrating the sample with NaOH (1 N) using phenolphthalein as an indicator and calculation by the given formula:

Titratable Acidity (mg/g) = (Volume of NaOH (mL) × Normality of NaOH × Equivalent weight of acid) / Weight of sample (g)

2.8. *Free radical scavenging potential*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) experiment was used to determine compounds' free radical scavenging capacity. The standard (ascorbic acid) and test samples were made by combining extract and ethanol, and absorbance was measured at 517 nm. The percentage

of antioxidant activity was calculated using the formula (AS - AT) × 100, where AS and AT are the absorbance of standard and test, respectively.

2.9. *Shelf-life and cost study*

Shelf life was determined by storing the product under specified conditions (4 °C) and regularly evaluating TA, sensory characteristics, and probiotic viability over time. The time during which the viable counts were $\geq 10^7$ CFU/mL with suitable retention of physicochemical and sensory properties was specified as the shelf life of the developed pickles. The cost was determined in Indian rupees (INR) through the incorporation of ingredient cost along with profit (15 %), processing cost (20 %), labor cost (15 %), and overhead cost (20 %).

2.10. *Analysis of data*

The data was analyzed by estimating the mean, standard deviation, standard error, paired t-test, and one-way ANOVA through MS Excel (version 2010). The significance of the data was calculated using the two-tailed T distribution, with p-values ranging from 0.05 to 0.001 to be considered statistically significant at confidence levels of 95 %. p-values <0.05, <0.01, and <0.001 were represented by *, **, and ***.

3. Results and Discussion

3.1. *Estimation of sensory characteristics and viable probiotic count*

Table 2. Sensory characteristics and viable probiotic count.

Product	Code	Appearance	Color	Taste	Texture	Odor	Overall rating	Viable Probiotic Count (CFU/g)
Brine (B)	B	7.56±1.8	7.04±1.5	7.71±0.9	7.60±0.8	7.46±1.4	7.75±1.2	0.00
Brine + Probiotic (BP)	BP	7.94±0.6**	7.25±0.9*	7.85±1.2*	7.65±0.7**	7.45±0.9*	7.88±0.7**	5.8 x 10^7± 92**
Brine + Probiotic + Banana peels (Fermented pickle-BPV3)	BPV 1	4.11±0.2**	6.33±0.8**	4.22±0.6**	6.56±0.8**	4.67±0.7**	4.56±0.8*	1.7 x 10^8± 75***
	BPV 2	7.56±0.5**	7.56±0.8**	7.67±0.7**	7.78±1.1*	7.89±0.8**	7.61±0.7**	2.2 x 10^8± 63***
	BPV 3	8.33±1.2*	7.89±0.7**	8.55±0.8**	8.00±0.8**	8.22±0.9*	8.67±1.1*	5.3 x 10^9± 56***

The developed products were estimated for sensory characteristics and viable probiotic count. The brine solution (B) inoculated with *L. rhamnosus* was mixed with varying proportions of banana peels in combination with other ingredients to develop synbiotic

pickles utilizing the synergistic combination of probiotic *L. rhamnosus* and prebiotics present in the peels (Table 2). A blend of spices and moderate levels of sugar were added to improve the organoleptic appeal of the final product, considering the natural taste and appearance of the banana peels, which may not be otherwise palatable. The cultures in BP, BPV1-3, were identified as *L. rhamnosus* owing to them being gram positive, catalase and oxidase negative, and capable of producing lactic acid upon sugar metabolism (data not shown). As observed in Table 2, BPV3 received the greatest sensory score amongst the synbiotic pickles with respect to the control (B). The proportion of ingredients used in this variation was observed to noticeably improve the characteristics visualized by the highest rating of appearance (8.33±0.4), color (7.89±0.7), taste (8.55±0.8), texture (8.00±0.8), odor (8.22±0.9) as well as the overall rating (8.67±1.1). Additionally, this variant (BPV3) was also seen to harbor the required number of viable probiotics (5.3×10^9 CFU/g) after 36 hours of fermentation as observed by growth in MRS agar, which was also maximum amongst all the developed variants. This was in concert with research that had documented the desired daily consumption range of probiotics between 10^8 and 10^9 CFU to be essential for obtaining health benefits [19]. Hence, BPV3 was further analyzed for its nutritional, health-promoting, and survival characteristics versus the control to understand the benefits that may be reaped through its consumption.

3.2. *In vitro determination of probiotic viability in a simulated human gastrointestinal environment*

The developed *Lactobacillus*-containing products (BP and BPV3) were subjected to treatment in a simulated human gastrointestinal environment (*in vitro*) to analyze the ability of probiotics in these products to tolerate the mentioned conditions, which is crucial for their effectiveness. It was demonstrated that the synbiotic pickle (BPV3) exhibited significantly improved survivability ($p < 0.05$) when exposed to created human gastric, bile, and intestinal digestion in contrast to BP that showed a marked decline in viable count over time (Table 3). The enhanced survivability can be attributed to the prebiotic properties of banana peel, as also indicated in previous studies, which provide a protective matrix and additional nutrients that support the viability of probiotics under these conditions, hence fostering a synbiotic environment [20]. This synergistic combination not only enhances the survival rate of the probiotics but also potentially amplifies their beneficial effects by ensuring successful colonization of beneficial *L. rhamnosus* in the small intestine. Consequently, the integration of banana peel with probiotics may lead to improved gastrointestinal health and overall well-being post utilization.

Table 3. Probiotic viability in simulated gastrointestinal conditions.

Treatment Duration (Hours)	Survival in an *in vitro* gastric environment ($O.D_{620\ nm}$)		Survival in an *in vitro* bile environment ($O.D_{620nm}$)		Survival in an *in vitro* intestinal environment ($O.D_{620nm}$)	
	BP	BPV3	BP	BPV3	BP	BPV3
24	0.61±0.06	0.85±0.05*	0.63±0.04	0.88±0.01*	0.64±0.06	0.89±0.04*

48	0.50±0.04	0.83±0.02*	0.58±0.03	0.79±0.02*	0.60±0.07	0.82±0.04*
72	0.48±0.07	0.79±0.03*	0.47±0.03	0.75±0.02*	0.58±0.05	0.81±0.03*

3.3. *Estimation of biochemical, physicochemical, and free radical scavenging properties*

The products were comprehensively evaluated for the presence of key biochemicals (nutrients and bioactive compounds), physicochemical properties (TA, TSS, and moisture), and free radical scavenging capacity to determine their contribution to overall dietary intake and potential health benefits. As indicated in Table 4, the synbiotic pickle (BPV3), containing brine, probiotics, and banana peels, showed increased carbohydrate (17.51 g/100 g) and fiber content (5.3 %) versus B and BP, mainly due to banana peel polysaccharides, including fructo-oligosaccharides, which may portray prebiotic properties as also visible through enhanced viable probiotics in the same (section 3.1). Moreover, BPV3 displayed a low protein (1.79 g/100 g) and fat (1.15 g/100 g) proportion, with the contents mainly attributed to the components of banana peels, making them approachable by those preferring protein and fat-restricted diets. Nonetheless, the omega-3 and omega-6 fatty acid content bestowed by the peels may help in limiting inflammation associated with metabolic diseases. Furthermore, BPV3 demonstrated significant presence of micronutrients, with 27.42 mg of magnesium, 79.55 mg of calcium, 29.41 mg of phosphorus, 0.47 mg of β-carotene, and 16.20 mg of vitamin C per 100 g ascribed by the peels of *Musa acuminata*. The synbiotic pickles also manifested a higher content of phytochemicals like phenols, flavonoids, alkaloids, and saponins compared to BP or B (Table 4), which may be due to the contribution of banana peels in the former along with the effect of fermentation that has previously been reported to increase the availability of phenols and other phytochemicals [21]. Additionally, the increased phytochemical content, especially phenols in BPV3, may favor the growth of probiotics, as demonstrated by enhanced *L. rhamnosus* viability in the current research, which is also in accordance with previous studies [22]. Additionally, BPV3 demonstrated notable free radical scavenging potential relative to BP and B, which may be endowed by the presence of the described phytochemicals (Table 4). Notwithstanding, BPV3 demonstrated a reduction in moisture and TSS versus B and BP owing to the effect of fermentation by *L. rhamnosus* on the peels, which along with a favorable TA due to acid production, may serve as a suitable matrix to favor probiotic survival, thereby helping them in exerting their beneficial action. Therefore, intake of BPV3, containing a synergistic combination of probiotics combined with banana peels, a typically discarded part of the fruit, may be conducive to gut health, immune function, and overall nutritional value.

Table 4. Biochemical, physicochemical, and free radical scavenging properties.

Nutrients (mg/100g)	Parameter	B	BP	BPV3
	Carbohydrate	1.54±0.05	1.47±0.04	17.51±1.89**
	Protein	0.21±0.02	0.20±0.01	1.79±0.1*
	Fat	0.14±0.01	0.12±0.02	1.15±0.007**

	Fibre	0.29±0.02	0.32±0.01	5.3±0.98**
	Iron	0.003	0.003	0.83±0.005*
	Calcium	0.403±0.01	0.387±0.01	79.55±6.78**
	Magnesium	0.196±0.02	0.187±0.01	27.42±1.23***
	Phosphorus	0.210±0.04	0.190±0.03	29.41±2.06***
	β-Carotene	0.003	0.004	0. 47±0.001**
	Vitamin C	0.001	0.001	16.20±1.22**
Phytochemicals	Total phenols (mgGAE/ 100g)	3.17 ± 0.32	3.35 ± 0.50	146.29 ±19.51***
	Flavonoids (mgQE/ 100g)	0.01	0.01	138.59 ±5.54***
	Alkaloids (%)	0.001	0.001	0.38 ± 0.03**
	Saponins (mg/100g)	0.215 ± 0.020	0.211 ± 0.013	3.47 ± 0.70 ***
Free radical scavenging	DPPH inhibition (% inhibition)	18.98±2.34	31.49±1.87**	88.09±2.55***
Physicochemical parameters	TA (mg/g)	0.001	0.64	7.68
	TSS (°Brix)	2	2	1
	Moisture (%)	97.25	96.13	64.2

3.4. *Determination of shelf life and cost of the developed synbiotic products*

The synbiotic pickles (BPV3), observed to be reservoirs of nutrients, bioactive compounds, and antioxidant properties, were assayed for their shelf life to evaluate their stability and safety throughout storage periods via estimation of viable probiotic numbers, sensory parameters, titratable acidity, and pH owing to the importance of these properties in governing the products' efficacy and quality. It was observed that BPV3 manifested a shelf life of 25 days at room temperature with retention of acceptable organoleptic characteristics, viable *Lactobacillus*, and acidity. Although a slight decline was noticed in the sensory scores beyond the 17th day, it remained within admissible limits till the 25th day. The titratable acidity remained within the desired range, peaking at 13.54 mg/g. Similarly, the pH levels stayed within safe limits, reaching 3.9 by the end of the 25 days. During the storage period, viable cell counts exhibited a slow and steady decrease, with a reduction of approximately 0.5 log order per week (Table 5). Nevertheless, the product maintained its probiotic characteristics throughout the 25-day period, with viable cell counts remaining well within the effective range of 10^6–10^7 CFU/g. Since the pickles were prepared without any preservatives, their shelf life may be further extended through the use of appropriate preservatives. The cost of the synbiotic pickles was calculated as INR 32.82 per 100 g which is lower than most of the synbiotic products available in the market (Table 5). The observed price of BPV3 was mainly due to the incorporation of the procured probiotic powder with the other ingredients being easily available and economical options. Since banana peels are typically considered a waste byproduct of the fruit, they were utilized at no cost in the final product. This makes the developed product highly affordable and accessible, allowing it to be easily prepared at the household level by individuals across all income groups. Therefore, BPV3 exhibited a stable shelf life and cost-effectiveness, making it accessible for people across various socio-economic backgrounds. Nonetheless, owing to the presence of live

probiotic strains, it should be consumed by immunocompromised and those with active infections only after medical consultation.

Table 5. Shelf life and cost of the developed synbiotic pickles.

Days	CFU/g	pH	TA	Sensory Score	Cost (INR)
Day 1	$2.2*10^8$	6.77	7.8	8.67	32.82
Day 7	$1.2*10^8$	5.45	9.7	8.50	
Day 14	$8.5*10^7$	4.82	10.9	8.42	
Day 21	$5.7*10^7$	4.04	13.1	7.63	
Day 25	$3.2*10^7$	3.90	13.54	7.21	

4. Conclusion

Food waste poses a global challenge with significant impact on the environment and economy. While valorization strategies exist, effective implementation is often limited. This study addresses the issue by developing a cost-effective synbiotic food product using discarded banana peels, promoting a zero-waste circular economy. Three formulations were tested, with the variant incorporating banana peels with probiotics in a brine solution (BPV3) showing the highest acceptability and enhanced probiotic growth due to prebiotic, nutritional, functional, and physicochemical properties bestowed by combining the peels with the other ingredients. It was observed to offer moderate carbohydrates as well as low protein and fat, making it suitable for calorie-restricted diets and managing conditions of metabolic imbalances. These products were noticed to manifest functional benefits like free radical scavenging potential owing to them being appreciable sources of key vitamins, minerals, and phytochemicals along with minimum cost and stable shelf life. Therefore, this sustainable and affordable solution may aid in promoting food waste utilization while offering significant health benefits.

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