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Original Research Article

Evaluating the Proximate Analysis, Antioxidant Activity and Probiotic Viability of Smoked Banana Probiotic Leather

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Abstract: This thesis investigates the proximate composition, antioxidant activity, and probiotic viability of smoked banana probiotic leather. Four samples were subjected to the proximate analysis, which included measurements of moisture, ash, protein, fat, and carbohydrate content: the non-smoked banana control (NSBC), smoked banana F1 (SBF1), smoked banana F2 (SBF2), and smoked banana F3 (SBF3). According to the findings, all samples had moisture content below 20%, with SBF3 having the greatest moisture level (3.36%) and SBF1 having the lowest (1.50%). SBF3 had the greatest ash content (0.46%), although there was a considerable variation in the ash content. The protein composition of the smoked and control samples varied significantly, ranging from 1.82% to 2.80%. The fat content remained low across all samples, while fiber content varied slightly, with SBF1 having the highest fiber content (4.17%). There were significant differences in the antioxidant activity between the samples, as determined by the DPPH radical scavenging activity, with SBF2 and SBF3 exhibiting higher activity. The product's antioxidant qualities may be strengthened by the smoking process and the addition of probiotics. There were no discernible variations between the groups in the probiotic viability ANOVA results, suggesting that Lactobacillus acidophilus was consistently viable throughout samples. This constancy is explained by the probiotic strain's stability and the homogeneity of the coating procedure. According to the study's findings, smoked banana probiotic leather can provide improved antioxidant qualities and nutritional advantages while preserving *Lactobacillus acidophilus*'s viability. To further enhance the product's quality and health advantages, future studies should concentrate on refining the formulation and processing methods. According to the research, smoked banana probiotic leather has the potential to be a useful meal with a number of health advantages.

Keywords: Smoked Banana, Probiotic Leather, Proximate Composition, Antioxidant Activity, DPPH Radical Scavenging Activity, Probiotic Viability, *Lactobacillus Acidophilus*.

INTRODUCTION

When it comes to its importance as a food crop, bananas are among the most popular fruits in the world, following rice, wheat, and maize. It is mostly grown in tropical and subtropical regions across more than 130 nations, with South-East Asia providing as its primary origin. The earliest bananas are said to have been produced in the Kuk valley of New Guinea in 8000 B.C., which is where the fruit is claimed to have originated 10,000 years ago. Later, these moved into the Philippines and other parts of Southeast Asia and the South Pacific, before spreading in all directions throughout the tropics (Denham *et al*., 2003). Bananas are easily perishable and instead of them becoming rotten or waste, the smoking technique is used. By making it a smoked banana, it can encourage against waste.

The idea of smoked bananas, or *pisang salai*, has its origins in culinary techniques, especially in areas where smoking is a popular way to preserve and flavor food. However, there is not enough preliminary research on smoked bananas. In certain parts of the country, smoking is still a common custom in Sri Lanka that is used to promote the ripening of bananas (Maduwanthi & Marapana, 2019). Smoked is a type of food made from ripe bananas preserved by drying. Drying can be done by drying under the sun and can also use artificial drying. In

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this era of technology, many drying methods have been used by using drying machines, among which is oven drying. After it is ready to be smoked banana, it can be used as any kind of product such as fruit leather.

Fruit leather is nutritious and a tasty addition to meals or in-between snacks among children and even adults. It is a chewy dried fruit product. The fruit is pureed, some seasonings and sweeteners can be added and then the puree is spread in a very thin layer on a drying rack or tray. This technique covers a healthy, portable snack option and they prevent fruits from spoiling, especially the overripe or remaining fruits. The fruit leathers are healthier products compared to other fruit products that contain essential nutrients such as minerals, vitamins, antioxidants, dietary fiber and carbohydrates among them. Also, texture is another critical aspect in acceptance of a product by the customers and depends on the drying circumstances 2 and the techniques used. (Diamante *et al*., 2014). Nowadays, people have an awareness about health so that fruit leather can be a functional food.

Foods that offer more health advantages than basic nourishment are known as functional foods. They include nutrients, bioactive chemicals, or other components that provide health benefits, lowering the risk of disease or encouraging a healthy lifestyle. The idea that food may significantly enhance general well-being and avoid chronic diseases is the basis of the functional food concept. In the 1980s, government authorities in Japan started approving foods that had been shown to improve public health, which gave rise to the idea of functional foods (De Jong *et al*., 2007). Functional foods can be defined as nutrient-dense foods such as fruits, vegetables, nuts, seeds, and grains, as well as foods enriched with vitamins, minerals, probiotics, or fiber.

Probiotic foods can be classified as functional foods, which are accepted by food professionals as foods that are designed and have properties other than their nutritional value to the consumer. The foods discussed above are also protective in that they prevent diseases, heart and nutrient related conditions, and encourage growth. That is, some of these foods can be taken regularly as part of the diets and might even enhance the general health of the person. Meanwhile, probiotic foods have traditionally been prepared with dairy ingredients, and it is characterized as live microorganisms that provide the host with health advantages when given in suitable amounts. However, due to an increase in vegetarians and the number of people who are lactose intolerant or allergic to milk proteins, there is currently a need for non-dairy diets that are focused on fruits and vegetables.

Fruits, vegetables, and drinks high in antioxidants are beneficial to human health since they can reduce the risk of several diseases and prevent diseases like cancer and cardiovascular disease. According to da Silva *et al*., (2010), antioxidants are substances that shield cells from the oxidative damage caused by reactive oxygen species, including superoxide, single oxygen, peroxyl radicals, hydroxyl radicals, and peroxynitrite.

The food industry relies highly on the viability of probiotic bacteria in food since it has an unbreakable connection to consumers' health and well-being. There are variations in the sensitivity of different microbe species to several factors such as substrate pH, temperature, dryness of the gastrointestinal system, and post-acidification in the fermentation process. The most interesting probiotic species found during testing was Lactobacillus acidophilus, which 3 showed significant levels of hydrophobicity and tolerance to bile and gastric fluid. (Vinderola, C.G., Reinheimer, J.A., 2003.)

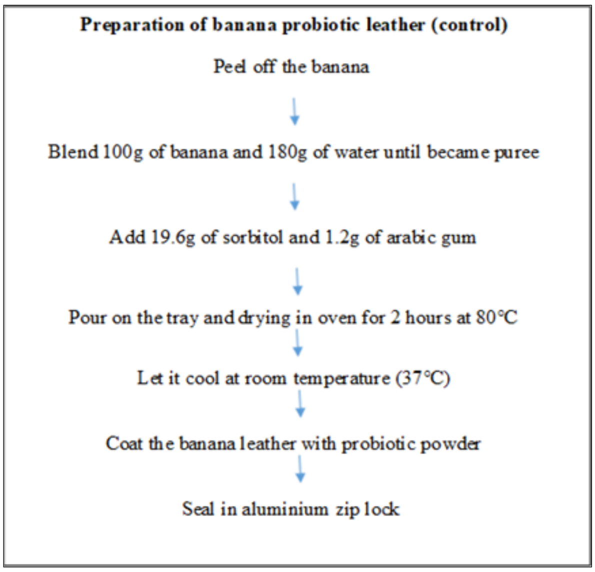
METHODOLOGY

Preparation of Control Banana Leather Probiotic

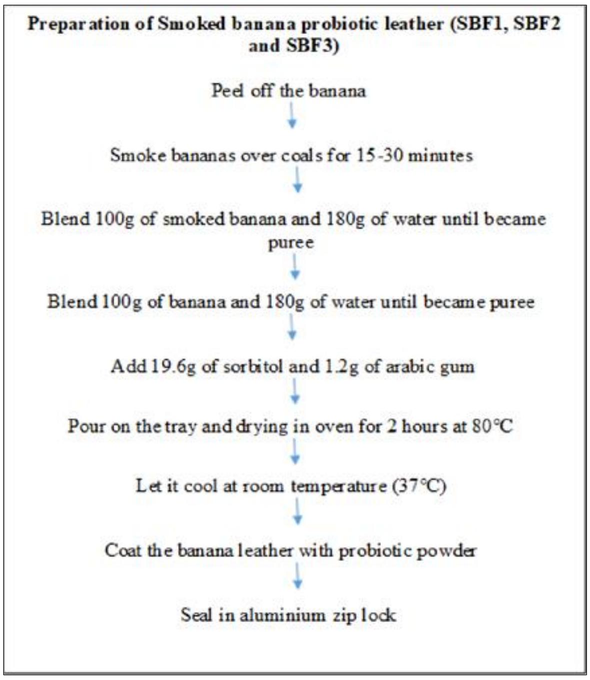
To prepare a control which is a banana probiotic leather, start by removing the banana peel with a knife and cutting the banana in half. Then, grind the bananas into a puree using a blender. Next, pour the banana puree onto a tray and spread it to form a thin layer. Place the tray in a heated oven and dry it for 2 hours and 30 minutes at the lowest temperature. Once the smoked banana leather has dried, remove it from the oven and let it cool at room temperature. Cooling is a vital step to prevent heat damage to probiotics, which can reduce their viability. After the leather has cooled, coat it with probiotic powder.

Preparation of Smoked Banana Fruit Leather with Probiotic

The first step in preparing is removing the banana peel using a knife and cutting the banana in half. Once the banana is cut in half, arrange the banana on the smoke rack and smoke for 15 to 30 minutes for both sides over a slow heat. The smoking process involves burning wood, which generates heat and produces smoke containing compounds with antibacterial properties. This imparts a unique flavor and offers a degree of preservation (Hagos, 2021). Burning the banana is a critical step that contributes to the development of the desired flavor because excess smoking turns into burning. After the bananas are ready to be smoked, make the bananas into a puree by grinding them using a blender. Next step, pour the banana puree on the tray and spread it to form a thin layer. Put it in a heated oven and dry it for 2 hours 30 minutes at the lowest temperature. After the smoked banana leather has been dried, remove it from the oven and let it cool at room temperature. Cooling is a vital step to prevent heat damage to probiotics that can reduce their viability. Then, coat the cooled smoked banana leather with probiotic powder.



Process flowchart for banana probiotic leather



Process flowchart for smoked banana probiotic leather

Poximate Analysis

The fat content, water activity, moisture, ash content, and total carbohydrate content of the smoked banana probiotic leather samples were analyzed in duplicate using standard procedures (AOAC, 2010).

Moisture Content

The moisture content was determined by oven-drying at 105 °C (AOAC, 967.08). Moisture content is calculated using the following formula:

$$A = \frac{[(W1 - W2)]}{W1} \times 100$$

Where A is moisture, W1 is the initial weight of the samples (before drying) and W2 is the weight of the samples after drying.

Ash Content

The total ash content was evaluated by the gravimetric method of incineration in a muffle furnace at 550 °C (AOAC, 942.05). The percentage of total crude ash was calculated using following equation.

$$Crude\ ash(\%) = \frac{[W1 - W2]}{W1} \times 100$$

Where W1 = weight of samples after ashing.
W2 = weight of samples before ashing.

Protein Content

The total protein content was determined by the Kjeldahl method and the conversion factor 6.25 used to convert into percent crude protein.

Fat Content

The crude fat content was measured by continuous extraction in a Soxhlet apparatus using hexane as solvent (AOAC, 2003.06). The fat content of smoked banana probiotic leather samples was calculated as following equation.

$$Fat\ (\%) = \frac{(m1 - m2)}{m0} \times 100$$

Where m0 = weight at the start of the analysis in g
m1 = weight of empty extraction beaker with boiling stones in g
m2 = weight of extraction beaker with fat after drying in g.

Carbohydrate Content

The total carbohydrate content of the samples was determined by adding the percentage values of hydration, ash, protein, and fat. The total value of the addition will be subtracted from 100%. The formula is as follows:
Carbohydrates (%) = 100 – [Moisture (%) + Ash (%) + Protein (%) + Fat (%) + Fibre (%)]

Fibre Content

The fibre content was determined by Gerhardt method using fibrebag. The percentage of total fiber crude was calculated using following equation.

$$\%\ CrudeFibre = [(C - A) - (D - E)]/B \times 100$$

Blank Value E = D – F

Meaning:
A = Mass FibreBag in g
B = Mass Sample weight in g (has to be adjusted according to dry content)
C = Mass Crucible and dried FibreBag after digestion in g
D = Mass Crucible and Ash in g
E = Blank Value of the empty FibreBag in g
F= Mass Crucible in g

Antioxidant Analysis

The antioxidant analysis of smoked banana probiotic leather was evaluated by using DPPH radical scavenging activity (Coklar & Akbulut, 2017). Briefly, a 0.1 mM solution of DPPH was prepared in methanol. Sample extracts were diluted to various concentrations (50–500 µg/mL), and 1 mL of each sample was mixed with 2 mL of the DPPH solution. The mixture was incubated in the dark for 30 minutes at room temperature, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. DPPH radical scavenging activity was calculated using the following equation.

$$\%Inhibition = \left(\frac{A\ control - A\ sample}{A\ control}\right) x100\%$$

Probiotic Viability

In this analysis, the pour plate method was used to determine the probiotics' viability. The researchers compared different inoculation methods and found that the pour plate method provided a relatively higher viable count than the spread plate method (Pyar, 2014).

$$\frac{CFU}{g} = \frac{(Number\ of\ colonies\ x\ Dilution\ factor)}{Sample\ weight\ (g)}$$

Gram Staining

To identify the morphological structure of lactic acid bacterial isolates, Gram staining was applied to each isolate using the procedure described in World Journal of Microbiology Biotechnology, 1992. Every isolate was used to create a bacterial smear on a clean, autoclaved slide, which was then allowed to air dry. After 30 seconds of washing away extra stain with distilled water, one drop of crystal violet was put next to the heat fixing. Following the addition of grams of iodine solution to the smear for 30 seconds, the iodine solution was repeatedly rinsed off with 95% ethyl alcohol, drop by drop, to remove any remaining stain. After safranin staining the slides for 30 seconds, the excess stain was removed with distilled water. The slides were then cleaned with distilled water once more. The slides were air dried and observed under compound microscope.

RESULT AND DISCUSSION

Proximate Analysis of Smoked Banana Probiotic Leather

Proximate analysis of smoked banana probiotic leather including moisture, ash, protein, fat, and carbohydrate are shown in Table 4.2. The analysis of moisture content for the 4 samples (non-smoked banana control, NSBC; smoked banana F1, SBF1; smoked banana F2, SBF2 and smoked banana F3, SBF3) showed the moisture content below 20%. The moisture content for SBF1 did not have significant (p>0.05) difference with control sample. The moisture content of the fruit leather was found to be a critical factor affecting its texture and shelf life. The study reported moisture content ranging from 10% to 20%, depending on the type of fruit and drying method used (Sukasih & Widayanti, 2022). The highest moisture content was recorded on SBF3 (1.88%), whereas the sample SBF1 had the lowest moisture content (0.10%). Higher moisture content in fruit leather SBF3 may be attributed to the banana without any preservative. Banana has a good moisture holding capacity (Joardder *et al*., 2013). This finding may be explained by the high pectin (a soluble dietary fiber) content of bananas.

Table 1: Proximate analysis of smoked banana probiotic leather

Percentage (%)	Control (NSBC)	F1 (SBF1)	F2 (SBF2)	F3 (SBF3)
Moisture	1.44 ± 0.22	1.50 ± 0.10	1.61 ± 0.14	3.36 ± 1.88
Ash	0.35 ± 0.01	0.31 ± 0.03	0.28 ± 0.01	0.46 ± 0.04
Protein	1.82 ± 0.01	2.01 ± 0.06	2.04 ± 0.14	2.80 ± 0.09
Fat	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Fibre	3.69 ± 0.32	4.17 ± 0.58	3.20 ± 0.10	3.88 ± 0.55
Carbohydrate	92.69 ± 0.51	91.99 ± 0.58	92.84 ± 0.30	89.48 ± 2.26

The ash content in samples varies, with the highest ash content observed in SBF3 (0.46%) and the lowest in SBF2 (0.28%). The significant differences in ash content between the control and the treatments (SBF2 and SBF3) indicate that the formulation and smoking process can influence the ash content. The study found that the ash content increased with the addition of carrageenan, highlighting the impact of specific ingredients on the nutritional quality of fruit leather (Setiaboma *et al*., 2019).

Protein, whose primary purpose is to provide enough of the amino acids needed for nutrition, is a necessary dietary component for both human and animal survival (Pugalenthi, M *et al*., 2004). The crude protein content of smoked banana probiotic leather ranged from 1.82% to 2.80%. The significant differences in protein content between the control (NSBC) and the smoked banana treatments (SBF1, SBF2, SBF3) indicate that the smoking process and the variations in the formulations effectively increased the protein content. The study found that protein-fortified mango leather had a protein content of 11.36g/100g (11.36%), which is significantly higher than the protein content in smoked banana probiotic leather's samples (Jethva *et al*., 2024). The fortification process and drying conditions played a crucial role in achieving this high protein content.

The fat content in across all the different treatments significantly difference ($p<0.05$). The study found that increasing the concentration of seaweed significantly decreased the fat content of the fruit leather (Ayustaningwarno *et al*., 2024). This aligns with findings, where the fat content remains low, indicating that certain additives or processes can maintain or reduce fat content in fruit leather.

The results from proximate analysis recorded that the fiber content in samples varies slightly across the different treatments. The highest fiber content is observed in SBF1 (4.17%), while the lowest is in SBF2 (3.20%). The variations in fiber content can be attributed to the different formulations and the smoking process used in each treatment. This research highlighted that fruit leathers are a convenient alternative to fresh fruits, since fiber is necessary in diet because it absorbs water and gives the intestines roughage, which aids in intestinal transit, the samples' variety of fiber contents is beneficial (Ibeji, C. C., 2011). The study emphasized the importance of dietary fiber in fruit leather, which is consistent with the fiber content observed in the samples.

Indicating that there is no significant difference ($p>0.05$) in carbohydrate content between SBF1 and the control group. This suggests that the smoking process and formulation changes in SBF1 did not significantly affect the carbohydrate content. Similarly, the p-value is greater than 0.05, indicating no significant difference in carbohydrate content between SBF2 and the control group. The slight variations in formulation for SBF2 did not lead to a significant change in carbohydrate content. The p-value is less than 0.05, indicating a significant difference ($p<0.05$) in carbohydrate content between SBF3 and the control group. This suggests that the formulation changes in SBF3, such as the absence of sorbitol and Arabic gum, significantly impacted the carbohydrate content.

Antioxidant Analysis (DPPH Radical Scavenging Activity)

The significant differences observed in DPPH activity among the sample groups highlight the effectiveness of the treatments in altering the antioxidant properties of the samples. Specifically, the smoked banana probiotic leather (SBF2 and SBF3) showed increased DPPH activity with higher concentrations, indicating a strong response to the treatment. Research has shown that banana-based products can serve as effective carriers for probiotics. For instance, a study on probiotic-loaded banana leathers demonstrated that incorporating probiotics like *Bacillus coagulans* and *Lactobacillus acidophilus* into banana leathers can enhance their functional properties (Niro *et al*., 2022).

The development of smoked banana probiotic leather not only leverages the natural antioxidant properties of bananas but also enhances them through the smoking process and probiotic incorporation. This combination can potentially offer a product with improved health benefits, including enhanced antioxidant activity and probiotic support. The study discussed how different processing methods, including smoking, can impact the antioxidant activity of food products. They highlighted that natural-source antioxidants are preferred over synthetic ones due to their health benefits (Gulcin & Alwasel, 2023).

Table 2: ANOVA of Antioxidant Analysis (DPPH radical scavenging activity)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.035897	3	0.011966	4.593202	0.016735	3.238872
Within Groups	0.041681	16	0.002605			
Total	0.077578	19				

Probiotic Viability

The F-statistic (0.841) is less than the critical value (2.866), and the p-value (0.480) is greater than 0.05. This indicates that there are no significant differences in probiotic viability between the groups. The results showed that the probiotic viability of *Lactobacillus acidophilus* in smoked banana fruit leather is consistent across different samples. This could be due to the uniformity in the probiotic coating process and the stability of the probiotic strain in the product. A study on the viability of probiotics in commercial dairy drinks found that probiotics can maintain their viability under various storage conditions (Liew *et al*., 2022). Similarly, it demonstrated that *Lactobacillus acidophilus* in acidophilus milk remained viable during frozen storage and retained its functional properties (Yurliasni *et al*., 2024). Additionally, the presence of prebiotic compounds in bananas, such as fructo-oligosaccharides, supports the growth and activity of probiotic bacteria (Rajyalakshmi *et al.*, 2019). These findings support the stability of Lactobacillus acidophilus in different food matrices, aligning with the results.

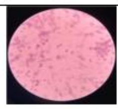
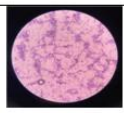
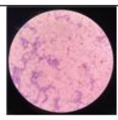
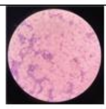
Table 3: ANOVA of probiotic viability

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.71E+23	3	1.57E+23	0.841044	0.480385	2.866266
Within Groups	6.72E+24	36	1.87E+23			
Total	7.20E+24	39				

Gram Staining

Under a microscope, *Lactobacillus acidophilus* appears purple because it is a Gram-positive bacterium, meaning it retains the crystal violet stain used in the Gram staining procedure. Its thick peptidoglycan coating in the cell wall, which is characteristic of Gram-positive bacteria, is the cause of this characteristic (Pyar, 2014).

Table 4: Gram staining of *Lactobacillus acidophilus* in smoked banana probiotic leather

Name	**Gram Staining**
a) **Lactobacillus acidophilus in NSBC**	
b) **Lactobacillus acidophilus in SBF1**	
c) **Lactobacillus acidophilus in SBF2**	
d) **Lactobacillus acidophilus in SBF3**	

CONCLUSION

The proximate analysis emphasizes how crucial the product's moisture, ash, protein, fat, fiber, and carbohydrate composition are in assessing its nutritional value. The study on smoked banana probiotic leather demonstrates that the product can maintain the viability of *Lactobacillus acidophilus*, a key probiotic strain, across different samples. The uniformity in probiotic viability suggests that the coating process and the stability of the probiotic strain are effective. This indicates the possibility of smoked banana probiotic leather as a functional food and is consistent with other studies on the persistence of probiotics in different food matrices. Prebiotic substances found in bananas also promote the growth and activity of probiotic bacteria, which adds to the product's overall health advantages.

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