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Nima Jose

Department of postharvest
 management, Kerala
 Agricultural University,
 Thrissur, Kerala, India

Maya T

Pineapple Research Station,
 Vazhakkulam, Kerala, India

Saji Gomez

Department of postharvest
 management, Kerala
 Agricultural University,
 Thrissur, Kerala, India

Effect of substrates on yield and physicochemical properties of *Nata de Pina*: Valorization strategy for low-grade fruits of pineapple (*Ananas comosus* (L.) Merr.) var. *Mauritius*

Nima Jose¹, Maya T², Saji Gomez³

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ABSTRACT

Pineapples are extensively cultivated around the world and processed into a plethora of products. The concomitant waste generated causes serious environmental concerns due to inadequate disposal and thus presents wide opportunities for novel product development and other valorization strategies. *Acetobacter* fermentation pathway of off-grade pineapples yields nata de pina, a remarkable biopolymer and acclaimed dessert. This research evaluates the effect of pineapple substrates (peel and juice) of Mauritius variety with varying dilution (1:2 and 1:3) on various physico-chemical properties and yield of nata de pina. *Nata* derived from pineapple peels diluted with twice the amount of water gave the highest yield (28.03 %), wet weight (14.01 g) and had the maximum thickness (9.27 mm) after 14 days of incubation. Substrate variation had a significant effect on total soluble solids of nata and fermentation medium. Dilution factor rather than substrate variation had a pronounced effect on acidity of nata de pina while pH of the nata showed a stable trend throughout various fermentation conditions. The findings indicate that pineapple peel waste serves as a more efficient and sustainable substrate for nata production than pineapple juice, which also gave sub-optimal yields, highlighting how off-grade pineapples can undergo efficient waste valorization. This edible film made of bacterial cellulose is characterized by exceptional mechanical strength, biodegradability, hydrophilic nature, and ultrafine network structure with innumerable applications across packaging and biomedical fields.

Keywords: *Nata de pina*, Pineapple, Waste valorization, Bacterial cellulose, Sustainability, Fermentation, *Acetobacter*, Mauritius pineapple

Introduction

Pineapple, belonging to bromeliaceae family is one of the widely cultivated fruit crops in tropics and subtropics, sought out for its nutritional richness and excellent quality juice (Ancos *et al.*, 2016)^[1]. Pineapple fruits are generally consumed as such or processed into canned pineapple slices, wine, juice, jams, and other confectionary items further highlighting its market presence. Furthermore, worldwide production and cultivated area of pineapple fruit have shown an upward trend from 1994 to 2023, emphasizing its demand in food and beverage industries (FAO, 2024)^[2]. Therefore, an escalation in waste generation is inexorable owing to the growing public health cognizance and elevated production levels. The non-edible parts such as crown, core, leaves, stems, and peels constituting about 80% of pineapple fruits are eliminated as waste during processing, transportation, and storage whereas waste generated after processing consisting of crown, peels, core, and pomace account for more than 60 % of the total fruit weight (Zainal Alam *et al.*, 2020; Sarangi *et al.*, 2022)^[4, 3]. Fruits that fail to meet established grading standards such as minimum total soluble solids content, uniformity in size and shape, and absence of defects etc. are often excluded from both international and domestic markets as well as processing chains, leading to significant underutilization and resource wastage in horticulture sector. Inadequate handling and disposal of these rich organic wastes lead to their decomposition, emitting potent greenhouse gases exacerbating climate change, and contributing to eutrophication of waterbodies endangering aquatic ecosystems, thus impacting the overall quality of environment (Rabiu *et al.*, 2018; Sial *et al.*, 2024)^[5, 6].

Multiple studies conducted on characterization of pineapple waste revealed higher quantities of sugars, carbohydrates, fibers, organic acids, and phenolic compounds making them valuable substrates for valorization processes (Khedkar *et al.*, 2017; Zain *et al.*, 2021)^[7, 8]. Leveraging this rich nutrient composition in processing residue, fermentation serves as an efficient and versatile pathway for valorization. *Nata de pina* or bacterial cellulose is a remarkable biopolymer and an acclaimed dessert synthesized and secreted by *Acetobacter xylinum* grown in a medium containing pineapple juice and necessary nutrients (Almeida *et al.*, 2013)^[9]. It

Corresponding Author:

Nima Jose

Department of postharvest
 management, Kerala
 Agricultural University,
 Thrissur, Kerala, India

exhibits distinct physico-chemical characteristics that render it a versatile ingredient for incorporation into a diverse array of food items, such as yogurt, salads, and pastries. Bacterial cellulose possesses unique properties including high purity, crystallinity and mechanical strength which extend its utility beyond desserts. Wang *et al.*, (2017)^[10] elucidated that the wide range of physical and mechanical attributes of bacterial cellulose makes it applicable across various fields such as tissue engineering, biomedicine, nano fluidics, wearable technology, functional foods, cosmetics, and bio composite materials.

While the general principles of pineapple waste valorization and *Nata de pina* production are established, there remains a discernible gap in specific research focusing on the detailed utilization of off-grade fruits of pineapple variety Mauritius' for *nata de pina* synthesis and the comprehensive characterization of its physicochemical properties and yield. A similar study has been conducted by Rojsuntornkitti *et al.*, (2020)^[11] on the effect of various concentrations of ammonium sulphate on characteristics of *nata* produced from pineapple cultivar Pattavia. Beyond that, studies have been focused on effect of other variables such as fermentation time, nitrogen, and carbon source concentration etc. (Mulawi & Kristina, 2019; Syukri *et al.*, 2025)^[12, 13] under different non-species-specific processing conditions (Sutanto, 2012; Khanagoudar & Narayanaswamy, 2016)^[15, 14]. Given that the changes in fermentation conditions will have significant effect on important *nata* characteristics, it becomes relevant to explore the effect of varying substrate type and concentration of pineapple variety Mauritius. Mauritius is a mid-season cultivar, commonly used for direct table consumption and holds great significance, by being predominantly grown in parts of India, such as Meghalaya and Kerala where Asia's largest pineapple market is situated. In this regard, the authors had conducted a study on physico-chemical characterization of processing waste of Mauritius pineapple revealed higher lignocellulosic and sugar content, making it ideal for *nata de pina* production (Jose *et al.*, 2024)^[16]

This research aims to bridge the scientific gap by investigating the effect of processing residue of pineapple variety Mauritius on the yield of *nata de pina*, evaluating the physicochemical properties (e.g., thickness, weight, pH, acidity, total soluble solids) of *nata de pina*

produced. Research on *nata* production conditions has significant industrial implications, primarily in optimizing production for increased efficiency and product quality. Understanding factors like substrate, temperature, pH, and nutrient levels allows for tailored processes that boost yield, reduce costs, and enhance product characteristics like texture and shelf life of *nata de pina*.

2. Materials and Methods materials required for fermentation

Off-grade (due to size and quality non-compliance) pineapple fruits of variety Mauritius, which are rejected and is unsuitable for processing, export, or local market sale were collected from Asia's largest pineapple market, Vazhakkulam. Various chemicals of pure grade such as ammonium sulphate, glacial acetic acid and sucrose were purchased from Sisco research laboratories pvt. Ltd. Polypropylene bottles (7 cm x 5.3 cm) used for fermentation were purchased from local markets.

2.2. Elemental analysis of pineapple peels

Peels of pineapple variety Mauritius was analysed for its elemental composition to determine C/N ratio using CHNS analyzer. 0.1 g of powdered and dried pineapple peels were used for this analysis.

3. Preparation of bacterial inoculum and nata starter

Bacterial inoculum of *Acetobacter xylinum* culture (NCIM 2526) was procured from the National Collection of Industrial Microorganisms (NCIM), Pune, Maharashtra. Freeze dried culture (Figure 1) was revived by emptying the content into 2-5 ml of *Acetobacter suboxydans* media prepared using the ingredients (Figure 2) (Table 1). One or two loopful of culture was streaked into *Acetobacter suboxydans* agar media and incubated at 28 °C for five days (Figure 3). It was then sub-cultured to obtain pure cultures, which were transferred to tomato agar slants for maintenance (Jagannath *et al.*, 2011)^[17].

For *nata* starter preparation, *Acetobacter xylinum* maintained on tomato agar slants were inoculated into sterilized Hestrin and Shramm media (Hestrin *et al.*, 1954)^[18]. The inoculated media was incubated statically at 30 °C for 7 days.



Figure 1. Freeze-dried *Acetobacter xylinum* (NCIM 2526) in glass ampoule

Table 1. Composition of *Acetobacter suboxydans* media

Ingredients	Quantity required
Sorbitol	5.0 g
Yeast extract	0.5 g
Distilled water	100 ml
Agar	2 g



Figure 2. Activation of *A. xylinum* in *A. suboxydans* broth medium



Figure 3. *Acetobacter xylinum* culture maintained on agar slants

4. preparation of substrates for *Nata* production

The experiment was conducted in Completely Randomized Design (CRD) with each treatment carried out in five replicates. *Nata de pina* production was performed as per the procedure described by (Jose *et al.*, 2024) [16]. Fruits were decrowned, cleaned and washed thoroughly with potable water after sanitizing with 120 ppm sodium hypochlorite solution for 15 minutes. The fruits were peeled and cut into pieces; juice was extracted using a fresh juice extractor (Sujatha Powermatic juicer). The peel was processed in an electric blender (Panasonic Mixer-Grinder MX AV425) for uniform blend. The juice/peel extract was diluted with distilled water in 1:2 and 1:3 ratio and used as the substrate for *nata* production. This media was mixed with ammonium sulphate (0.5 %) and sucrose (10%). The pH was adjusted to 3.5 and sterilized. The sterilized substrate was inoculated with starter culture (40 % of the concentration) under aseptic condi-

tions, covered with muslin cloth and incubated at ambient conditions for 14 days. The treatments included were S₁- Pineapple juice diluted with water in 1:2 ratio, S₂- Pineapple peel diluted with water in 1:2 ratio, S₃- Pineapple juice diluted with water in 1:3 ratio and S₄ - Pineapple peel diluted with water in 1:3 ratio.

5. Harvest and purification of *Nata* de pina

Upon completion of the fermentation period, the *Nata de pina* layer was carefully harvested. The harvested *nata* sheets were made to undergo a rigorous purification process involving repeated washing with distilled water to remove residual fermentation medium, unreacted sugars, and acetic acid etc. This purification step is crucial for obtaining pure *nata de pina* suitable for further characterization and application.

6. Physicochemical characterization of *nata* de pina

Physico-chemical characteristics of *nata de pina* such as thickness, weight, T.S.S, pH, acidity, yield, and T.S.S of the fermentation medium were analyzed. Thickness of *nata* sheet was measured at four different positions using a vernier caliper. Values were averaged and expressed as mm. Weight of *nata* (g) was measured using a weighing balance after draining off the water from *nata* for 5 minutes. Yield of *nata* was measured in g/ml and expressed in percentage. The harvested *nata* was washed, cut into pieces, and crushed using mortar and pestle until a fine paste was obtained. Total soluble solids were recorded using ERMA handheld refractometer (0-32 °B) and expressed in degree brix (°B). The pH of the *nata* was measured using an ECPHTUTOR-S CyberScan pH Tutor Meter (U.S.). Acidity of *nata* was estimated by acid-base titration using a phenolphthalein indicator (AOAC, 1998) [19].

7. Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA), Group means were compared and grouped based on Duncan's Multiple Range Test (DMRT), where significant effects were found.

2. Results and Discussion

Table 2. Effect of substrates on yield and physico-chemical properties of *nata de pina* after 14 days

Treatment	Thickness (mm)	Wet weight (g)	Yield (%)	TSS (°B)	pH	Acidity (%)	Final TSS of the fermentation medium
S ₁	9.48 ^a	12.66 ^b	25.33 ^b	10.16 ^c	2.84 ^a	1.66 ^a	18.56 ^b
S ₂	9.27 ^a	14.01 ^a	28.03 ^a	11.58 ^b	2.68 ^{bc}	1.66 ^a	16.24 ^d
S ₃	8.33 ^b	11.50 ^c	23.01 ^c	10.16 ^c	2.67 ^c	0.89 ^b	18.12 ^c
S ₄	8.23 ^{bc}	12.83 ^b	25.66 ^b	14.58 ^a	2.75 ^b	0.89 ^b	20.92 ^a
CD	0.12	1.07	2.15	0.70	0.07	0.47	0.40

3.1 Thickness of *nata* de pina

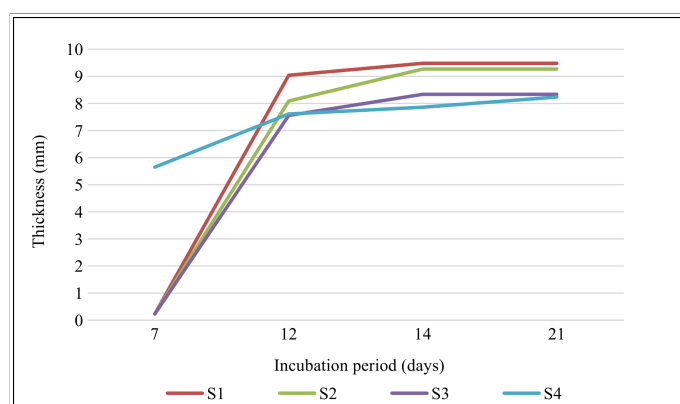
The thickness of *nata de pina* exhibited distinct trends influenced by both substrate type and concentration, indicating the varied metabolic responses of *Acetobacter xylinum* to different fermentation conditions (Figure 5-9). Notably, initial *nata* formation differed significantly across treatments. While no discernible growth was detected in S₁, S₂, and S₃ during the first seven days, *nata* formation commenced on the fourth day in S₄, achieving a thickness of 5.65 mm. Earlier initiation of *nata* formation in S₄ suggests a convenient and optimally balanced nutrient profile in the peel extract of pineapple variety Mauritius as outlined by (Jose *et al.*, 2024) [16] for the initial proliferation of *Acetobacter xylinum* compared to other treatments. This is further supported by our elemental analysis (CHNS) of the pineapple

peels, which revealed a high carbon-to-nitrogen (C/N) ratio of 84.72. Such a high C/N ratio typically indicates abundant carbon sources relative to nitrogen, which can strategically favor the carbon-intensive process of cellulose biosynthesis over rapid microbial biomass accumulation during the initial lag phase (Sari *et al.*, 2020) [20] further elucidated that *Acetobacter xylinum* exhibits a higher substrate utilization rate in media with lower glucose content, whereas the rate of cellulose formation is enhanced in the presence of higher glucose concentrations. This kinetic behavior aligns with our observations. Despite a slower initial growth phase, the substrates diluted with twice amount of distilled water (S₁ and S₂), characterized by a higher immediate availability of fermentable glucose, and ultimately supported the maximum observed *nata de pina* thickness (9.48 mm and 9.27 mm respectively).

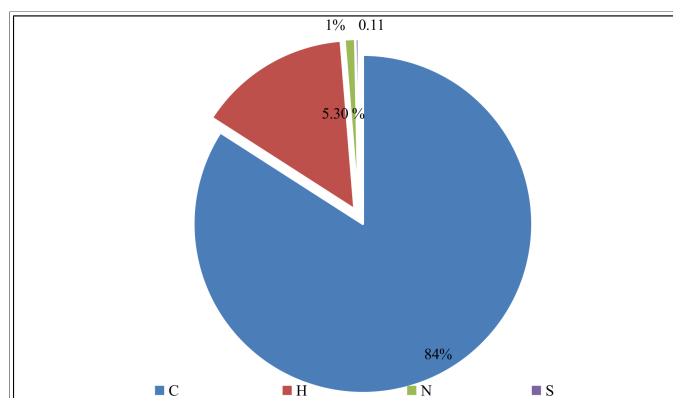
Table 3. Effect of substrates and incubation period on thickness of *nata de pina*

Treatments	7 th day	12 th day	14 th day	21 st day
S ₁	0.22 ^e	9.04 ^a	9.48 ^a	9.48 ^a
S ₂	0.22 ^e	8.09 ^{bc}	9.27 ^a	9.27 ^a
S ₃	0.22 ^e	7.56 ^c	8.33 ^b	8.33 ^b
S ₄	5.65 ^d	7.61 ^c	7.86 ^{bc}	8.23 ^{bc}

The final thickness measurements on day 21 showed negligible differences from day 14 values across all four treatments ($p < 0.05$) (8.33 mm and 8.23 mm in S₃ and S₄, respectively), suggesting an optimum fermentation time of approximately 14 days under these experimental conditions (Table 3). This finding partially aligns with (Juwita *et al.*, 2020)^[21], who reported an optimum fermentation time of 21 days for *nata de pina* produced from peels, highlighting that optimal fermentation duration can vary depending on specific processing parameters. Beyond this optimal period, factors such as nutrient depletion, accumulation of inhibitory metabolic byproducts, or autolysis of bacterial cells may limit further cellulose synthesis (Ye *et al.*, 2019)^[22].

**Figure 4.** Effect of substrates on increase in thickness of *nata de pina* during incubation period

Moreover, it was observed that the texture of *nata* in S₄ was significantly harder than all other treatments. This increased rigidity in peel-derived *nata* can primarily be attributed to the inherent chemical composition of pineapple peels, which possess a higher content of lignocellulosic fibers and other complex carbohydrates (Roha *et al.*, 2013)^[23]. These macromolecular components likely facilitate the formation of a denser and more intricately interconnected bacterial cellulose network during *A. xylinum* biosynthesis, leading to a more robust final texture. While some strains of *Acetobacter xylinum*, such as BPR2001, have been reported to produce cellulase enzymes that can decrease the degree of polymerization and consequently the mechanical strength of bacterial cellulose, particularly under certain pH conditions (Tahara *et al.*, 1997)^[24], our observed increase in hardness in S₄ suggests that any such cellulase activity in our specific strain and conditions was either minimal, or its impact was significantly overshadowed by the enhanced network formation facilitated by the peel substrate's composition (Figure 4). Conversely, the comparatively looser texture observed in the diluted juice treatment (S₃) might also be influenced by factors like lower initial substrate concentration leading to a less dense cellulose network, or potentially a relative increase in the impact of any endogenous cellulase activity relative to cellulose synthesis in that specific diluted environment.

**Figure 5.** CHNS composition of pineapple peels of variety Mauritius

Furthermore, the fermentation medium utilizing pineapple peels (S₂, S₄) exhibited a deeper yellow hue. This is consistent with the natural pigmentation inherent to pineapple peels, and while *Acetobacter xylinum* is known for urease activity that can produce ammonia and influence pH affecting the color (Syafiqah *et al.*, 2020)^[25], the predominant factor contributing to this deeper yellow in the final product is likely the direct transfer and stability of these natural pigments.

3.2. Weight and yield of *nata de pina*

The highest fresh weight and yield were observed in S₂, while the lowest was recorded in S₃. This observation aligns with Khanagoudar & Narayanaswamy (2016)^[14] who reported a decrease in the fresh weight of *nata* with increasing dilution of pineapple juice. Our findings collectively suggest that S₂ provided an optimal balance of fermentable sugars and other nutrients, promoting maximal bacterial cellulose synthesis. In contrast, while dilution generally reduces yield due to lowered fermentable sugar availability (as clearly seen in the very low yield of S₃), the yields in S₁ and S₄ appear to be sub-optimal compared to S₂, but for different underlying reasons. S₁, as a less diluted juice medium, may have encountered conditions *beyond* the optimal range for *Acetobacter xylinum* activity, potentially due to factors such as substrate saturation or an elevated rate of gluconic acid production. While these are not necessarily inhibitory to the point of complete cessation, it could shift the pH rapidly away from the ideal range for peak cellulose synthesis, as noted by Chen *et al.* (2011)^[26]. The yield of S₄, which utilizes pineapple peels at a certain concentration, while being superior to S₃, falls short of S₂. This could be attributed to the inherent characteristics of the peel substrate (e.g., initial total sugar concentration, the rate of complex carbohydrate hydrolysis, or the presence of non-sugar components) that, at this specific concentration, did not perfectly match the highly optimized conditions found in S₂. Thus, the similar yields of S₁ and S₄ represent convergence at a sub-optimal level, driven by distinct limitations related to their specific substrate types and concentrations. In addition to that, no significant statistical differences were observed between the weight and yield of *nata* layers obtained from S₁ and S₄ ($p < 0.05$). A significant finding of our study is the comparatively higher yield obtained from pineapple peels (S₂, S₄) compared to pineapple juice (S₁, S₃), underscoring the efficiency and potential for valorization of pineapple peels as a substrate for *nata* production. These results are consistent with Yovita *et al.* (2020)^[27], who also reported superior yields of *nata de pina* from pineapple fruit peel (12.02 %) compared to other parts of the fruit, including the core and juice. It can also be seen that yield of *nata de pina* from honey variety of pineapple was

less than that of Mauritius variety for the same dilution. The enhanced yield from pineapple peels can be attributed to their distinct carbohydrate profile and complex matrix. While pineapple juice predominantly contains simple sugars, peels are rich in both fermentable sugars and complex carbohydrates. Preliminary experiments by Embuscado *et al.* (1994)^[28] highlighted that sucrose, a predominant sugar in both pineapple peel and core extracts yielded significantly higher bacterial cellulose (5.25 g/L) compared to glucose (1 g/L). This suggests that the specific sugar composition of pineapple peels, potentially with a more favorable sucrose-to-glucose ratio, could contribute to better bacterial fermentation. Moreover, the higher cellulose content inherently present in pineapple peels can undergo partial acid hydrolysis during the prolonged fermentation process, forming glucose (Ifeyanyi *et al.*, 2024)^[29], which then becomes available for bacterial utilization. This continuous release of fermentable sugars from complex carbohydrates provides a sustained energy source, potentially leading to higher overall cellulose yields.

Our study achieved remarkably high yields, averaging 12.5 g per 50 mL of medium (equivalent to 250 g/L). This significantly surpasses previously reported yields for bacterial cellulose production, particularly when considering the nature of the primary substrate. Sari *et al.* (2021)^[20] reported yields of 1.5-1.4 g/L from a coconut water medium that was modified and optimized to maintain a carbon-to-nitrogen (C/N) ratio of 5.39. In contrast, our primary carbon source, the

raw pineapple peel, exhibited an inherent C/N ratio of 84.72. While the actual C/N ratio of our complete fermentation medium (containing added nutrients like ammonium sulphate and sucrose) would differ, the dramatically higher C/N ratio of the foundational peel substrate itself strongly indicates its substantial carbon richness. This inherent abundance of carbon, readily available for metabolism and cellulose synthesis as discussed in Section 3.1, provides a compelling explanation for the substantially higher cellulose production observed in our peel-based system, despite the differences in substrate preparation and medium optimization strategies compared to other studies.

The reduction in yield observed in S₃ and S₄ compared to S₁ and S₂, is consistent with the findings of Sasithorn (2008)^[30], who reported that while an initial increase in dilution (e.g., from 1:1 to 1:2) could significantly increase yield, further increases in dilution beyond an optimum point led to a reduction. This indicates that while pineapple peels are an efficient substrate, an optimal concentration of fermentable sugars and other essential nutrients is critical for maximizing bacterial cellulose production, and excessive dilution can still limit overall yield, even with the benefits of the peel matrix. The abundant and potentially more complex nutrient matrix in the fermentation medium of treatment S₄ likely contributed to the development of a faster and harder-textured bacterial cellulose layer, as indicated by initial growth observations.



Figure 6. *Nata de pina* obtained and fermentation medium with juice diluted in 1:2 ratio



Figure 7. *Nata de pina* obtained and fermentation medium with pineapple peel diluted in 1:2 ratio

3.3. T.S.S, pH and acidity of *Nata de pina*

The total soluble solids (TSS), pH, and titratable acidity of the *nata de pina* samples varied notably across the different treatments, reflecting the dynamic metabolic activity of *Acetobacter xylinum* during

fermentation.



Figure 8. : *Nata de pina* obtained and fermentation medium with pineapple juice diluted in 1:3 ratio

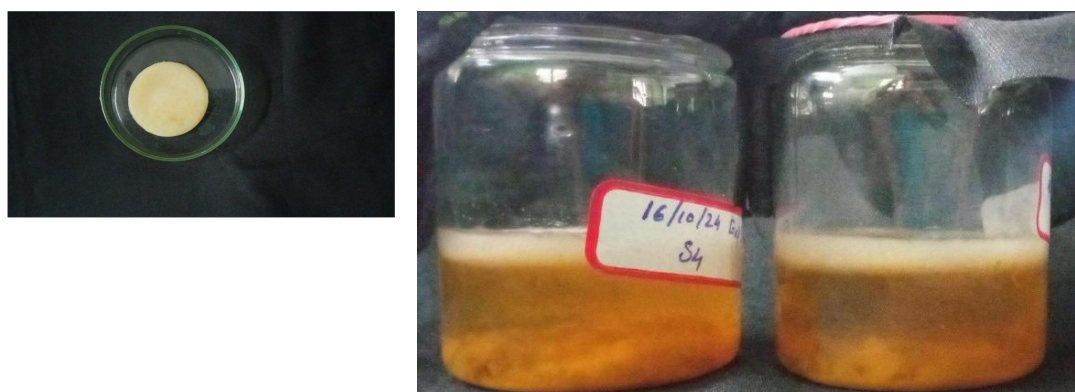


Figure 9. *Nata de pina* obtained and fermentation medium pineapple peel diluted in 1:3 ratio

Total Soluble Solids (TSS): The highest TSS was observed in S₄, while the lowest levels were recorded in S₃ and S₁. Notably, TSS of S₃ and S₁ were significantly lower than in S₂ (Table 2). Interestingly, treatments using pineapple peel as a substrate generally maintained higher TSS values than those using pineapple juice as substrates, which could be attributed to the inherent differences in the initial physicochemical composition of the substrates. Higher residual TSS of the fermentation medium values in certain treatments, particularly S₄, might suggest that not all fermentable solids were fully utilized by the bacteria during fermentation. Conversely, fermentation media with the lowest final TSS value, such as S₂, demonstrated the highest yield, indicating more efficient conversion of available soluble solids into bacterial cellulose. This inverse relationship underscores the importance of optimizing substrate concentration for maximal substrate utilization and yield. Although, the treatments with sub-optimal yields (S₁ and S₄) did not exhibit this inverse relationship. Total soluble solids (TSS) of *nata* made from pineapple juice consistently measured 10 across both dilution levels, with the fermentation medium TSS also remaining stable at 18. In contrast, pineapple peel exhibited a different response when *nata* TSS increased from 11 at 1:2 dilution to 14 at 1:3 dilution, while its fermentation medium TSS similarly rose from 16 to 20. Simply stated, while pineapple juice *nata* and medium TSS remained constant when dilution increased from 1:2 to 1:3, pineapple peel *nata* and fermentation medium TSS both rose with the higher dilution.

The breakdown of juice and peel matrix which is possibly catalyzed by endogenous peel enzymes, microbial enzymes (if present), or the acidic environment created by gluconic acid production (Verschuren *et al.*, 2000) [31], would release new soluble sugars and oligosaccharides into the medium. Consequently, the final TSS does not solely reflect unutilized initial fermentable sugars but also comprises newly solubilized components, making a direct correlation between TSS re-

duction and cellulose yield more intricate than with simple sugar media.

pH: The pH levels of the *nata de pina* varied significantly across treatments (Table 2). The highest pH was recorded in S₁ (2.84), while the lowest was observed in S₃ (2.67). There were no significant statistical differences in pH between S₂, S₃, and S₄ ($p > 0.05$). A notable trend observed was a decrease in the pH of *nata de pina* with increasing dilution when pineapple juice was used as the substrate. This could be explained by the reduced buffering capacity of more diluted juice, allowing the gluconic acid produced by *Acetobacter xylinum* to cause a more pronounced drop in pH. Conversely, an increase or stabilization in pH was observed when pineapple peels were used as the substrate. This contrasting behavior is likely due to the variation in the composition of both substrates. Pineapple peels possess a higher concentration of lignocellulosic and fiber components (Roha *et al.*, 2013) [23], which may provide a greater buffering capacity against the acidification caused by gluconic acid production, or be metabolized differently, resulting in a less drastic pH reduction. Comparing our results to other studies, *nata de pina* produced from Pattavia pineapple juice, which had a pH of ≈ 3.8 , resulted in a product pH as low as 1.5 (Rojsumtornkitti *et al.*, 2020) [11]. This difference from our Mauritius pineapple juice treatments (final pH ≈ 2.7 -2.8) suggests potential varietal distinctions. The Mauritius pineapple, with its higher initial pH (5.43) might possess a greater buffering capacity or a different sugar profile that influences the rate or extent of gluconic acid accumulation, leading to a slightly less extreme final pH, despite robust acid production.

The consistent final pH values across all our treatments (juice vs. peel, different dilutions) in the range of 2.6-2.8, despite varying initial pH values and substrate types, highlights the strong acid-producing capacity of the *Acetobacter xylinum* strain used and its tendency to drive the medium to a specific acidic equilibrium (Vandamme *et al.*, 1998) [32]. Although these final pH values are slightly below the gen

erally reported optimum range for *A. xylinum* growth, the high cellulose yields observed in our study (especially S₂) suggest that the strain is highly tolerant to such acidic conditions, or that the critical period for optimal activity occurs before the pH drops to these extreme levels. Furthermore, this low pH environment is crucial for inhibiting the growth of most contaminating microorganisms, contributing to the safety and stability of the *nata de pina* product. Optimal conditions for *nata* production involve temperatures ranging from 25 °C to 30 °C and a slightly acidic pH, favoring bacterial proliferation. (Talawar *et al.*, 2015)^[33]

Acidity: S₁ and S₂ demonstrated similar titrable acidity levels (1.66%), as S₃ and S₄ (0.89%) (Table 2). No significant differences were observed between treatments using pineapple juice and those using pineapple peel as the primary substrate ($p > 0.05$). This suggests that the dilution ratio, rather than the specific type of substrate (juice vs. peel), played a primary role in determining the final acidity levels of *nata de pina*. This is corroborated by the findings of Thankappan & Anitha (2018) ^[34] who reported a titrable acidity of ~1.2 for *nata* produced from medium where pineapple juice was used as an acidulant. The concentration of fermentable sugars that are converted to gluconic acid by *Acetobacter xylinum* appears to be the dominant factor influencing the final titrable acidity. This implies that while peel-based substrates offer benefits in terms of yield and texture, the acidic profile can be modulated primarily through control of initial substrate concentration.

3. Conclusion

This study comprehensively evaluated the impact of different pineapple juice and peel concentrations on the yield, and physicochemical properties of *nata de pina*. Our findings strongly advocate for the valorization of peels and juice of off-grade pineapple fruits which are rejected due to non-compliance of quality characteristics as a highly efficient and sustainable substrate for bacterial cellulose production. This is primarily attributed to the complex carbohydrate matrix of peels, including beneficial sucrose levels and the potential for acid hydrolysis of lignocellulosic components, providing a sustained and diverse carbon source for *Acetobacter xylinum*. The distinct physicochemical properties of variety Mauritius demonstrate the tunability of *nata de pina* by manipulating substrate type and concentration. This has critical implications for developing diversified *nata* products with specific attributes for various food applications. The observed pH and acidity profiles, strongly influenced by dilution rather than solely substrate type, provide valuable insights for process control and ensuring product safety and stability. Future research should focus on a more detailed characterization of the precise sugar profiles and other nutrient compositions of both pineapple juice and peel extracts throughout the fermentation process to elucidate the exact metabolic pathways contributing to the observed differences in yield and texture. Further optimization of peel processing (e.g., enzyme pretreatment, different extraction methods) could potentially enhance fermentable sugar availability and further improve yields. This study provides a robust scientific basis for the sustainable production of high-quality *nata de pina* from pineapple variety Mauritius, contributing significantly to food waste reduction and the development of novel functional food ingredients.

4. Acknowledgements

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5. Declaration of interest statement

No competing interest was reported by the author(s)

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