

## Article

# Effects of Modified Atmosphere Packaging and Densified Dry Bacterial Cellulose on the Physicochemical and Microbiological Stability of Beneng Taro Flour during Storage

### Article Info

#### Article history :

Received July 16, 2026  
Revised August 17, 2026  
Accepted August 20, 2026  
Published August 30, 2026  
*In Press*

#### Keywords :

Beneng taro flour,  
modified atmosphere packaging,  
bacterial cellulose,  
moisture management,  
total plate count (TPC)

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**Abstract.** Beneng taro flour is a hygroscopic food powder susceptible to quality deterioration during storage. This study evaluated the effects of Modified Atmosphere Packaging (MAP) and densified dry bacterial cellulose on its physicochemical and microbiological stability. Five treatments were investigated: air (P1), 100% N<sub>2</sub> (P2), 100% O<sub>2</sub> (P3), 100% N<sub>2</sub> + bacterial cellulose (P4), and 100% O<sub>2</sub> + bacterial cellulose (P5) during 12 weeks of storage. Two-way ANOVA of log<sub>10</sub>-transformed Total Plate Count (TPC) showed significant effects of packaging treatment, storage time, and their interaction ( $P < 0.05$ ). At week 12, P2 and P4 exhibited the lowest TPC values, at  $263 \pm 116$  and  $387 \pm 131$  CFU/g, respectively. Moisture content was significantly negatively correlated with Log<sub>10</sub> TPC ( $r = -0.350$ ,  $P = 0.0186$ ), whereas ash content showed a positive but non-significant correlation ( $r = 0.281$ ,  $P = 0.0616$ ). Multiple linear regression showed that moisture and ash contents jointly explained 59.2% of the variation in Log<sub>10</sub> TPC ( $R^2 = 0.592$ ,  $P < 0.001$ ). Overall, nitrogen-based MAP was associated with improved microbiological stability of Beneng taro flour, while densified dry bacterial cellulose showed potential as a complementary moisture-management component.

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**1. Introduction**

Beneng taro (*Xanthosoma undipes*) is an underutilized tuber with potential for food applications. More broadly, taro flours and starches have attracted increasing interest because of their carbohydrate-rich composition, functional properties, and potential applications in gluten-free and functional food products [1-3]. Beneng taro flour has also been developed as an ingredient for various food products. The physicochemical characteristics of taro flour and starch, including their gelatinization, viscosity, rheological, gel-forming, and water-absorption properties, support their potential application in diverse food formulations [2-4]. However, maintaining the quality of taro flour during storage remains challenging because flour-based products are susceptible to moisture exchange with the surrounding environment.

Starch, the major component of taro flour, contains numerous hydroxyl ( $-OH$ ) groups capable of interacting with water molecules through hydrogen bonding. Consequently, starch-rich powders may adsorb moisture from the surrounding environment depending on storage conditions and package barrier properties [5-6]. Changes in moisture conditions during storage can affect powder stability, including flowability, caking behavior, physicochemical characteristics, and microbiological stability. Therefore, controlling the package microenvironment, particularly moisture and gaseous composition, is important for maintaining the quality of hygroscopic flour products during storage. Previous research on taro flour has demonstrated that packaging material can influence its storage stability. High-density polyethylene (HDPE), for example, has been reported to provide a longer estimated shelf life than polypropylene (PP), indicating the importance of package barrier characteristics in controlling quality deterioration [7].

Although conventional polymeric packaging provides a physical barrier between food and the external environment, passive packaging alone may not sufficiently regulate changes occurring within the package headspace during prolonged storage. Modified Atmosphere Packaging (MAP) offers an alternative strategy by modifying the gaseous composition surrounding the product. Depending on the food system, MAP can reduce oxygen availability and modify the conditions associated with oxidative reactions and microbial activity [8].

Nitrogen ( $N_2$ ), in particular, is widely used as an inert displacement gas because it can reduce the proportion of oxygen in the package headspace without reacting directly with the food matrix [9], [10]. Consequently, nitrogen-based atmosphere modification may contribute to limiting oxygen-dependent deterioration during storage. However, the effectiveness of MAP is influenced not only by the initial gas composition but also by the barrier characteristics of the packaging material and environmental conditions during storage.

Gas and water-vapor transmission through polymeric packaging can progressively modify the internal package environment [8],[11-12]. For hygroscopic powdered foods, moisture transfer is particularly important because changes in the moisture status of the product may influence physicochemical stability and microbial behavior. Thus, atmosphere modification alone may not provide complete control over the package microenvironment, creating a need for complementary moisture-management strategies. Active packaging provides an additional approach by incorporating components capable of interacting with the internal package environment. Among the materials potentially suitable for this purpose, bacterial cellulose has attracted considerable attention because of its distinctive structural properties.

Bacterial cellulose is a biopolymer produced by cellulose-producing bacteria and is characterized by a three-dimensional fibrillar network, high porosity, large surface area, and abundant hydroxyl groups capable of interacting with water molecules [13]. These properties make bacterial cellulose a promising material for moisture-management applications in food packaging. Previous work has demonstrated that drying and densification can produce compact dry bacterial cellulose while retaining structural and physicochemical characteristics relevant to moisture adsorption [9]. When incorporated into a permeable sachet without direct contact with the food, densified dry bacterial cellulose may function as a moisture-scavenging component by adsorbing water vapor present within the package headspace. Such an approach is particularly relevant for hygroscopic powdered foods, in which maintaining a stable internal moisture environment is important for preserving product quality.

From a packaging-system perspective, nitrogen-based MAP and densified dry bacterial cellulose may provide complementary functions. Nitrogen primarily modifies the gaseous environment by displacing oxygen from the package headspace, whereas bacterial cellulose may contribute to moisture management through water-vapor adsorption. Combining these two approaches therefore provides a potential strategy for simultaneously managing two important aspects of the package microenvironment: gaseous composition and moisture conditions. However, the magnitude of the contribution of each component may depend on storage duration, package permeability, residual headspace gases, product characteristics, and environmental conditions. Therefore, the effectiveness of their combined application needs to be evaluated experimentally rather than assumed to result from a synergistic mechanism. Previous studies on the storage stability of flour products have largely focused on conventional packaging materials, whereas MAP has been extensively investigated for fresh fruits and vegetables and other perishable food products [14-16].

Bacterial cellulose, meanwhile, has been widely investigated as a biomaterial for food and packaging applications because of its porous fibrillar structure and moisture-interaction characteristics [9], [13]. Nevertheless, studies examining the combined application of modified atmosphere conditions and densified dry bacterial cellulose as a moisture-management component for hygroscopic tuber flour remain limited. In particular, information regarding their application to Beneng taro flour and their effects on both physicochemical characteristics and culturable microbial load during storage is still lacking.

Accordingly, this study investigated an integrated packaging approach combining modified atmosphere conditions with densified dry bacterial cellulose as a moisture-management component for Beneng taro flour. Five packaging treatments air as the control, 100% N<sub>2</sub>, 100% O<sub>2</sub>, 100% N<sub>2</sub> with bacterial cellulose, and 100% O<sub>2</sub> with bacterial cellulose were evaluated during storage to determine how differences in packaging conditions affected product stability. The study specifically aimed to evaluate changes in moisture content, ash content, and Total Plate Count (TPC) under the different packaging treatments and storage periods.

In addition, Pearson correlation and multiple linear regression analyses were employed to examine the statistical associations between physicochemical characteristics and microbial load. Through this approach, the study provides information on the potential application of modified atmosphere conditions and densified dry bacterial cellulose for maintaining the physicochemical and microbiological stability of hygroscopic Beneng taro flour during storage.

## 2. Experimental Section

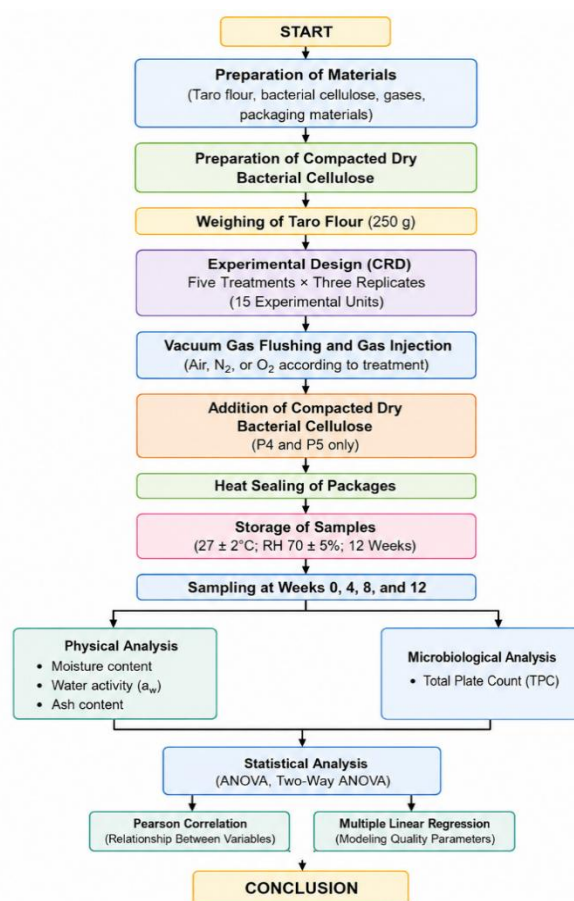
### 2.1. Materials

The study began with the preparation of Beneng taro flour, densified dry bacterial cellulose, food-grade gases, and packaging materials. The flour was subsequently packaged according to five treatments differing in gas atmosphere and the presence or absence of densified dry bacterial cellulose (Table 1).

**Table 1** Experimental Design Matrix

| Treatment | Gas            | Densified dry bacterial cellulose |
|-----------|----------------|-----------------------------------|
| P1        | Air (Control)  | No                                |
| P2        | N <sub>2</sub> | No                                |
| P3        | O <sub>2</sub> | No                                |
| P4        | N <sub>2</sub> | Yes                               |
| P5        | O <sub>2</sub> | Yes                               |

Packaged samples were stored under controlled environmental conditions, and changes in their physicochemical and microbiological characteristics were evaluated during storage. The resulting data were analyzed using Two-Way Analysis of Variance (ANOVA), Pearson correlation analysis, and multiple linear regression, as appropriate for the respective response variables. The research workflow is illustrated in Figure 1.

**Figure 1.** Schematic flowchart of the research.

Beneng taro (*Xanthosoma undipes*) flour with an initial moisture content of approximately 12% was used as the primary material. Dry bacterial cellulose was produced through fermentation using *Komagataeibacter xylinus* (formerly *Acetobacter xylinum*), followed by purification with 1% NaOH, washing to neutral pH, drying at 80 °C, and densification using a hydraulic press. The preparation of densified dry bacterial cellulose followed the procedure reported by Ardiani et al. [9].

Food-grade nitrogen ( $N_2$ ) and oxygen ( $O_2$ ) were used for atmosphere modification. Packaging was performed using 80- $\mu$ m polyethylene (PE) bags. The equipment included a vacuum gas-flushing machine, impulse sealer, analytical balance, drying oven, microbiological incubator, and supporting equipment for moisture and ash analyses. Each package contained 250 g of Beneng taro flour. Independent packages were prepared for each treatment x storage-time x replicate combination; therefore, each package represented a separate experimental unit. Three independent packages were prepared for each treatment at each storage time ( $n = 3$ ).

For treatments P4 and P5, approximately 5 g of densified dry bacterial cellulose was enclosed in a permeable sachet and positioned inside the package without direct contact with the flour. For treatments P2–P5, the package headspace was evacuated using a vacuum gas-flushing machine and subsequently flushed with the designated gas before sealing. Treatments P2 and P4 were flushed with  $N_2$ , whereas P3 and P5 were flushed with  $O_2$ .



**Figure 2.** Packaging procedure for Beneng taro flour under modified-atmosphere conditions with densified dry bacterial cellulose.

Treatment P1 was packaged under normal atmospheric conditions and served as the control. Nitrogen was selected because it is widely used as an inert displacement gas in modified-atmosphere packaging to reduce the oxygen concentration within the package headspace and thereby limit oxygen-dependent deterioration. Recent reviews describe  $N_2$  as a major component of MAP systems because of its low reactivity and ability to displace oxygen from the package environment [8]. Oxygen was included as a comparative atmosphere to evaluate the physicochemical and microbiological responses of Beneng taro flour under oxygen-rich conditions; this constituted part of the experimental design and therefore does not require an external citation.

The densified dry bacterial cellulose incorporated into P4 and P5 was intended to serve as a moisture-management component. This application is supported by the porous fibrillar structure and abundant hydroxyl groups of bacterial cellulose, which provide potential interaction sites for water molecules and support its use in food-packaging applications [16-17]. Recent work specifically identifies bacterial cellulose as a promising food-packaging material because of its fibrous network, hydrophilic functional groups, and capacity for modification toward moisture-related applications [17].

All samples were stored at  $27 \pm 2$  °C and  $70 \pm 5\%$  relative humidity for 12 weeks. TPC was determined at weeks 0, 4, 8, and 12, whereas moisture and ash contents were determined at weeks 0, 4, and 8. Consequently, analyses involving physicochemical variables together with TPC were restricted to the common sampling points at weeks 0, 4, and 8.

## 2.2. Physicochemical and Microbiological Analyses

Moisture content and ash content were evaluated as physicochemical quality parameters. Moisture content was determined using the air-oven method according to AOAC Official Method 925.10, whereas ash content was determined gravimetrically according to AOAC Official Method 923.03. Moisture content was determined using the air-oven method according to AOAC Official Method 925.10, whereas ash content was determined gravimetrically according to AOAC Official Method 923.03 [18].

Microbiological quality was evaluated using Total Plate Count (TPC) according to ISO 4833-1:2013, which specifies colony enumeration at 30°C using the pour-plate technique. Microbiological quality was evaluated using Total Plate Count (TPC) according to ISO 4833-1:2013 [19]. TPC results were expressed as colony-forming units per gram (CFU/g). All measurements were performed in triplicate, and results are presented as mean  $\pm$  standard deviation (SD).

## 2.3. Statistical Analysis

TPC data were  $\log_{10}$ -transformed prior to inferential statistical analysis to reduce scale heterogeneity and improve conformity with the assumptions of parametric analysis. The transformed values were expressed as  $\log_{10}$  CFU/g, whereas descriptive TPC data are presented on the original CFU/g scale as mean  $\pm$  SD to facilitate microbiological interpretation. The effects of packaging treatment, storage time, and their interaction on  $\log_{10}$ -transformed TPC were evaluated using two-way ANOVA with replication at a 95% confidence level ( $\alpha = 0.05$ ).

Packaging treatment consisted of five levels (P1–P5), while storage time consisted of four levels (weeks 0, 4, 8, and 12). Each treatment–time combination consisted of three replicate observations ( $n = 3$ ). The effects of packaging treatment, storage time, and their interaction on moisture and ash contents were also evaluated using two-way ANOVA with replication at a 95% confidence level ( $\alpha = 0.05$ ). For these physicochemical variables, packaging treatment consisted of five levels (P1–P5), while storage time consisted of three levels (weeks 0, 4, and 8).

Statistical significance was established at  $P < 0.05$ . Pearson correlation analysis was performed to evaluate the linear associations among moisture content, ash content, and  $\log_{10}$ -transformed TPC. Because moisture and ash contents were measured only at weeks 0, 4, and 8, correlation analysis was restricted to these common sampling points. A total of 45 paired observations were included in the correlation analysis. Pearson's correlation coefficient ( $r$ ) was used to describe the direction and strength of the linear associations. Multiple linear regression analysis was subsequently performed to evaluate the joint statistical associations of moisture content and ash content with microbial load. The regression analysis included the same 45 paired observations from weeks 0, 4, and 8. The model was expressed as:

$$Y = a + b_1.X_1 + b_2.X_2$$

where  $Y$  represents  $\log_{10}$  TPC ( $\log_{10}$  CFU/g),  $X_1$  represents moisture content (%),  $X_2$  represents ash content (%),  $a$  is the intercept, and  $b_1$  and  $b_2$  are the regression coefficients. Model performance was evaluated using the coefficient of determination ( $R^2$ ), adjusted  $R^2$ , overall  $F$ -test, regression coefficients,  $t$ -statistics, and associated  $P$ -values. Multicollinearity between predictors was evaluated using the variance inflation factor (VIF). Regression coefficients were interpreted as statistical associations rather than evidence of direct causal relationships. Statistical analyses were performed using IBM SPSS Statistics version 26 and Microsoft Excel 2024.

### 3. Results and Discussion

#### 3.1 Total Plate Count (TPC)

Two-way ANOVA performed on  $\log_{10}$ -transformed TPC data showed that packaging treatment, storage time, and their interaction significantly affected the microbial load of Beneng taro flour ( $P < 0.05$ ; Table 2). Packaging treatment had a significant effect ( $F = 80.19$ ,  $P = 1.48 \times 10^{-18}$ ), while storage time exhibited an even stronger effect ( $F = 213.12$ ,  $P = 1.25 \times 10^{-24}$ ). A significant interaction between packaging treatment and storage time was also observed ( $F = 22.82$ ,  $P = 3.56 \times 10^{-14}$ ), indicating that changes in microbial load during storage differed among packaging treatments. The significant interaction also indicates that the effect of packaging treatment was dependent on storage duration; therefore, the treatment effects should be interpreted in relation to the observed temporal patterns rather than solely from the overall main effects.

**Table 2.** Results of Two-way ANOVA for  $\log_{10}$ -Transformed TPC of Beneng Taro Flour

| <i>Source of Variation</i> | <i>SS</i> | <i>df</i> | <i>MS</i> | <i>F</i> | <i>P-value</i>         | <i>F crit</i> | <i>Note</i> |
|----------------------------|-----------|-----------|-----------|----------|------------------------|---------------|-------------|
| Packaging treatment        | 11.1702   | 4         | 2.7926    | 80.1916  | $1.48 \times 10^{-18}$ | 2.6060        | Significant |
| Storage time               | 22.2651   | 3         | 7.4217    | 213.1232 | $1.25 \times 10^{-24}$ | 2.8387        | Significant |
| Treatment x Storage time   | 9.5359    | 12        | 0.7947    | 22.8195  | $3.56 \times 10^{-14}$ | 2.0035        | Significant |
| Within (Error)             | 1.3929    | 40        | 0.0348    | -        | -                      | -             | -           |
| Total                      | 44.3642   | 59        | -         | -        | -                      | -             | -           |

In general, all treatments showed an overall reduction in TPC during the 12-week storage period, although the magnitude and temporal pattern of the reduction differed considerably among treatments (Table 3). This observation is consistent with the significant treatment x storage time interaction obtained from the Two-way ANOVA. The reduction was particularly pronounced in the nitrogen-based treatments P2 and P4. P2 decreased from  $64,000 \pm 13,000$  CFU/g at week 0 to  $263 \pm 116$  CFU/g at week 12, whereas P4 decreased from  $60,667 \pm 7,572$  CFU/g to  $387 \pm 131$  CFU/g. In comparison, P1 and P3 retained higher microbial loads at week 12, reaching  $3,500 \pm 361$  and  $3,533 \pm 1,457$  CFU/g, respectively. P5 also showed a substantial decrease, reaching  $1,797 \pm 410$  CFU/g at week 12.

The observed differences among treatments may be associated with the combined influence of packaging atmosphere, moisture availability, and storage duration. Modified atmosphere packaging modifies the gaseous environment surrounding the food and may restrict conditions favorable for aerobic microbial activity, while active packaging components such as moisture scavengers can provide an additional mechanism for controlling the package microenvironment [8], [20-22]. The performance of MAP systems depends on the maintenance of the intended headspace composition, which may change during storage because of gas transfer through the packaging material [8], [10]. In low-moisture foods, microbial persistence is additionally influenced by factors such as water activity, storage temperature, and characteristics of the food matrix [8], [23-24]. Therefore, the observed reduction in culturable microbial load should be interpreted as the combined outcome of the packaging treatment and storage conditions rather than as evidence of a single antimicrobial mechanism.

The temporal profiles shown in Table 3 reveal substantial differences among packaging treatments. At week 4, the microbial load in P2 decreased sharply from  $64,000 \pm 13,000$  to  $2,703 \pm 887$  CFU/g, whereas P4 decreased more gradually from  $60,667 \pm 7,572$  to  $28,667 \pm 7,572$  CFU/g. By week 8, however, both nitrogen-based treatments exhibited markedly low microbial loads, with P2 reaching  $337 \pm 12$  CFU/g and P4 reaching  $417 \pm 121$  CFU/g. At the end of storage, P2 exhibited the lowest TPC ( $263 \pm 116$  CFU/g), followed by P4 ( $387 \pm 131$  CFU/g).

In contrast, the air control (P1) and oxygen treatment without bacterial cellulose (P3) retained the highest microbial loads at week 12, at approximately  $3.5 \times 10^3$  CFU/g. These differences support the significant treatment x storage time interaction identified by ANOVA. The relatively low TPC observed in the nitrogen-based treatments is consistent with the role of nitrogen as an inert displacement gas that reduces the availability of oxygen in the package headspace. Reduced oxygen availability may create conditions less favorable for aerobic microbial metabolism [25]. Nitrogen-based atmosphere modification has therefore been widely used as part of MAP systems to limit oxygen-dependent deterioration during food storage [20], [21], [26], [27]. Nevertheless, because the residual headspace  $O_2$  concentration was not periodically quantified in the present study, the observed reduction in TPC cannot be attributed quantitatively to oxygen displacement alone.

The comparison between P3 ( $O_2$  without bacterial cellulose) and P5 ( $O_2$  with bacterial cellulose) also provides useful evidence regarding the potential contribution of moisture management. At week 12, P5 exhibited a lower TPC ( $1,797 \pm 410$  CFU/g) than P3 ( $3,533 \pm 1,457$  CFU/g). Bacterial cellulose possesses a three-dimensional fibrillar network, high porosity, and abundant hydroxyl groups capable of interacting with water molecules [16], [17], [23], [28]. These structural characteristics provide a plausible basis for its function as a moisture-adsorbing material. Thus, the lower microbial load observed in P5 relative to P3 may be associated with the presence of densified dry bacterial cellulose; however, this interpretation should be considered cautiously because water activity and package relative humidity were not continuously monitored during storage.

Interestingly, P2 ( $N_2$  without bacterial cellulose) showed a slightly lower TPC than P4 ( $N_2$  + bacterial cellulose) at week 12 ( $263 \pm 116$  versus  $387 \pm 131$  CFU/g). Therefore, the present TPC data do not support the conclusion that adding bacterial cellulose to the  $N_2$  atmosphere necessarily produced a lower final microbial count than  $N_2$  treatment alone. Rather, both nitrogen-based treatments demonstrated strong reductions in microbial load, with different temporal patterns. The potential contribution of bacterial cellulose should therefore be evaluated together with the physicochemical results, particularly moisture-related parameters, rather than inferred solely from the final TPC values.

**Table 3.** Total Plate Count (TPC) of Beneng Taro Flour under Different Packaging Treatments during 12 Weeks of Storage

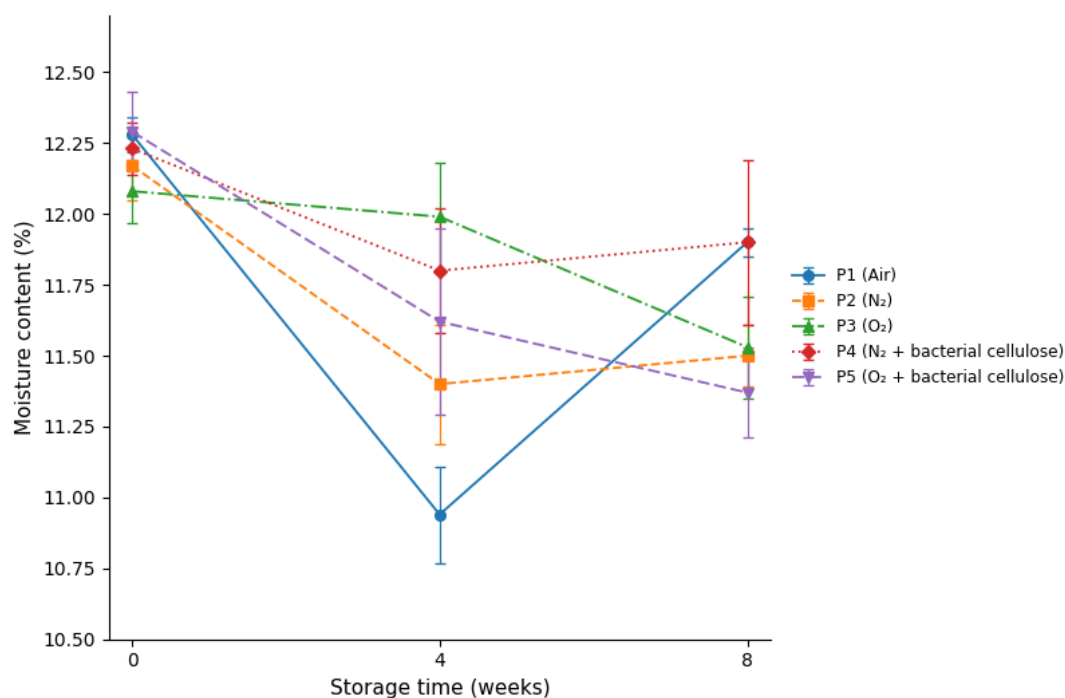
| Treatment | Storage time      |                   |                   |                    |
|-----------|-------------------|-------------------|-------------------|--------------------|
|           | Week 0<br>(CFU/g) | Week 4<br>(CFU/g) | Week 8<br>(CFU/g) | Week 12<br>(CFU/g) |
| P1        | 59,000 ± 8,660    | 35,000 ± 7,810    | 37,000 ± 2,646    | 3,500 ± 361        |
| P2        | 64,000 ± 13,000   | 2,703 ± 887       | 337 ± 12          | 263 ± 116          |
| P3        | 40,667 ± 9,504    | 24,667 ± 5,033    | 32,333 ± 3,512    | 3,533 ± 1,457      |
| P4        | 60,667 ± 7,572    | 28,667 ± 7,572    | 417 ± 121         | 387 ± 131          |
| P5        | 35,667 ± 8,327    | 30,000 ± 9,000    | 26,300 ± 11,676   | 1,797 ± 410        |

Overall, the progressive reduction in culturable microbial load during storage may reflect increasingly restrictive environmental conditions within the packaged flour matrix. In low-moisture foods, microbial viability and culturability are influenced by several interacting factors, including water activity, temperature, food matrix composition, physiological adaptation, and storage duration. Microorganisms may persist under low-water-activity conditions without active proliferation, and their recoverability by culture-based methods may decrease during prolonged storage [24], [29-33]. Accordingly, the decline in TPC observed in this study should be interpreted as a reduction in culturable microbial load, rather than direct evidence that microorganisms were completely eliminated from the product. Although the nitrogen-based treatments showed particularly low microbial loads, the underlying mechanism cannot be conclusively separated into the individual contributions of gas composition and moisture adsorption using the current dataset.

Headspace O<sub>2</sub> and N<sub>2</sub> concentrations, package relative humidity, and water activity were not continuously monitored throughout storage. Therefore, the present findings provide evidence that packaging treatment significantly influenced microbial stability but do not establish a direct causal mechanism for each packaging component. Future studies incorporating periodic measurements of headspace gas composition, relative humidity, and water activity would provide stronger mechanistic evidence regarding the respective contributions of atmosphere modification and bacterial cellulose to microbial stability.

### 3.2 Physical Characteristics: Moisture and Ash Content of Beneng Taro Flour

The physicochemical stability of Beneng taro flour during storage was evaluated based on changes in moisture and ash contents. Moisture content is an important quality parameter for dry food products because changes in product moisture may result from interactions among the food matrix, package headspace, packaging material, and surrounding environment. In polymeric food-packaging systems, the transfer of water vapor is influenced by the barrier properties of the packaging material as well as environmental conditions such as temperature and relative humidity [11]. Ash content, in contrast, represents the inorganic residue remaining after combustion of the organic components of the sample. The moisture and ash content profiles of Beneng taro flour under the different packaging treatments are presented in Figure 4 and Figure 5.



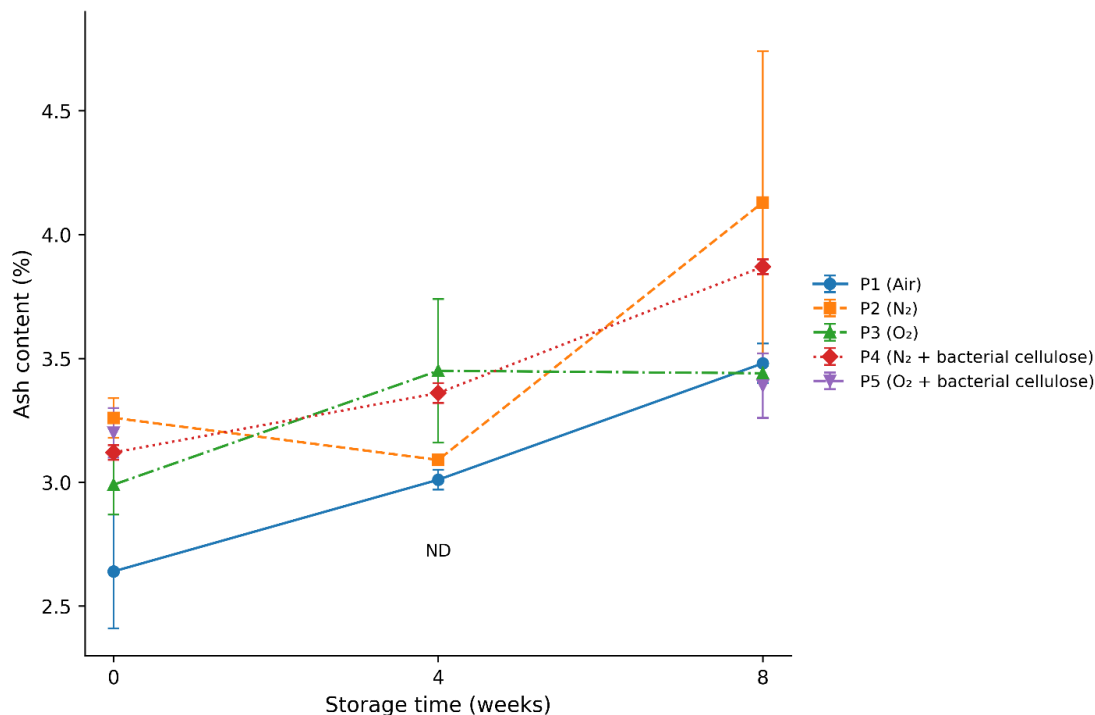
**Figure 4.** Changes in moisture content of Beneng taro flour under different packaging treatments during 8 weeks of storage. Values are expressed as mean  $\pm$  SD ( $n = 3$ ).

Two-way ANOVA showed that moisture content was significantly affected by packaging treatment ( $F = 11.49$ ,  $P < 0.001$ ) and storage time ( $F = 84.69$ ,  $P < 0.001$ ). However, the interaction between packaging treatment and storage time was not statistically significant ( $F = 1.96$ ,  $P = 0.086$ ). These results indicate that both packaging conditions and storage duration influenced the overall moisture level of the flour, although the temporal pattern of moisture change did not differ significantly among treatments.

Moisture content generally increased during the eight-week storage period, although the magnitude of the increase differed descriptively among treatments. In the air control (P1), moisture content increased from  $11.96 \pm 0.23\%$  at week 0 to  $13.10 \pm 0.27\%$  at week 8, representing the highest final moisture content among the treatments. P3 also showed a relatively pronounced increase, from  $11.62 \pm 0.35\%$  to  $12.84 \pm 0.22\%$ . In contrast, P4 and P5 showed comparatively lower moisture contents at week 8, reaching  $12.39 \pm 0.18\%$  and  $12.30 \pm 0.11\%$ , respectively, while P2 reached  $12.90 \pm 0.31\%$ . The comparatively lower moisture accumulation observed in the treatments containing densified dry bacterial cellulose, particularly P4 and P5, provides a plausible indication of its potential moisture-management function.

Bacterial cellulose is characterized by a three-dimensional fibrillar network, high surface area, porosity, and abundant hydroxyl groups, which contribute to its hydrophilic properties and interactions with water [34]. Previous characterization of compacted dry biocellulose derived from *Xanthosoma undipes* also provides direct support for the use of this material in the present experimental system [9]. These characteristics provide a physicochemical basis for interaction with water and support the potential application of densified dry bacterial cellulose as a moisture-management material. Nevertheless, the moisture response cannot be attributed exclusively to bacterial cellulose because moisture transfer in packaged foods is also influenced by the water-vapor permeability of the packaging material, temperature, relative humidity, and the resulting moisture-transfer conditions between the package and its environment [11].

Nitrogen primarily functions in MAP as an inert gas used to modify the package atmosphere rather than as a moisture-absorbing material [8]. In contrast, bacterial cellulose may contribute to moisture management through its hydrophilic structure and interaction with water [16]. The comparatively lower moisture contents observed in P4 and P5 may therefore be associated with the presence of densified dry bacterial cellulose. However, because the treatment  $\times$  storage-time interaction was not statistically significant, these descriptive differences should not be interpreted as evidence of a statistically confirmed synergistic interaction between the modified atmosphere and bacterial cellulose. Furthermore, package relative humidity and water activity were not continuously measured during storage; therefore, the specific mechanism by which bacterial cellulose affected moisture conditions within the package cannot be conclusively established from the present dataset.



**Figure 5.** Changes in ash content of Beneng taro flour under different packaging treatments during 8 weeks of storage. Values are expressed as mean  $\pm$  SD ( $n = 3$ ).

Ash content showed a different statistical pattern. Two-way ANOVA indicated a significant effect of packaging treatment on ash content ( $F = 7.74$ ,  $P < 0.001$ ), whereas the effect of storage time was not statistically significant ( $F = 2.80$ ,  $P = 0.077$ ). The interaction between packaging treatment and storage time was also not significant ( $F = 1.15$ ,  $P = 0.363$ ). Thus, differences in ash content were primarily associated with packaging treatment rather than a uniform storage-time effect. Ash values ranged from  $3.33\%$  to  $4.12\%$  during the observation period. The most pronounced increase occurred in P1, from  $3.59 \pm 0.18\%$  at week 0 to  $4.12 \pm 0.28\%$  at week 8. P3 similarly increased from  $3.47 \pm 0.25\%$  to  $3.90 \pm 0.23\%$ . In contrast, P4 remained comparatively stable, changing from  $3.44 \pm 0.19\%$  at week 0 to  $3.34 \pm 0.25\%$  at week 8.

P2 and P5 also exhibited relatively limited changes compared with P1 and P3. Because ash content represents the inorganic residue remaining after combustion of the organic matter, the observed changes should not be interpreted as evidence of mineral formation or degradation during storage. Rather, ash content is expressed as a proportion of sample mass; therefore, changes in the relative proportions of sample constituents may influence the apparent ash percentage. Because

protein, lipid, carbohydrate, and other proximate components were not measured longitudinally at each storage period, the compositional basis underlying the observed variation in ash content cannot be established from the present dataset.

Overall, the physicochemical results show that moisture content was significantly influenced by both packaging treatment and storage duration, whereas ash content was primarily influenced by packaging treatment. The comparatively moderate moisture accumulation observed in P4 and P5 supports the potential application of densified dry bacterial cellulose as a moisture-management component, although its independent effect cannot be conclusively established from moisture-content data alone. Importantly, moisture content should not be equated with water activity ( $a_w$ ). Water activity describes the availability of water relevant to microbial processes and is an important determinant of microbial growth and survival [35-36].

Peleg demonstrated the fundamental role of  $a_w$  in determining microbial growth rates and growth-initiation thresholds [24] while studies of low-moisture foods have shown that microbial survival is affected by water activity together with other factors such as temperature and food-matrix characteristics [24]. Therefore, the relationships between the measured physicochemical characteristics and microbial load were further evaluated using Pearson correlation and multiple linear regression analyses.

### 3.3 Correlation Analysis between Physicochemical Characteristics and Microbial Load

Pearson correlation analysis was performed to evaluate the linear relationships among moisture content, ash content, and microbial load expressed as  $\log_{10}$ -transformed Total Plate Count ( $\log_{10}$  TPC). Because moisture and ash contents were measured at weeks 0, 4, and 8, the correlation analysis was restricted to these common sampling periods. A total of 45 paired observations were included in the analysis.

**Table 4.** Pearson Correlation Coefficients among Physicochemical Characteristics and Microbial Load of Beneng Taro Flour

| Variables                  | Moisture Content ( $X_1$ ) | Ash Content ( $X_2$ ) | $\log_{10}$ TPC (Y) |
|----------------------------|----------------------------|-----------------------|---------------------|
| Moisture content ( $X_1$ ) | 1.000                      |                       |                     |
| Ash content ( $X_2$ )      | 0.663*                     | 1.000                 |                     |
| $\log_{10}$ TPC (Y)        | -0.350*                    | 0.281                 | 1.000               |

Note: Pearson correlation was calculated using paired observations from weeks 0, 4, and 8 ( $n = 45$ ). \* $P < 0.05$ .

Moisture content showed a statistically significant negative correlation with  $\log_{10}$  TPC ( $r = -0.350$ ,  $P = 0.0186$ ), representing a weak-to-moderate inverse association. This result indicates that, across the pooled experimental observations, higher moisture-content values tended to occur together with lower culturable microbial loads. However, this relationship should not be interpreted as evidence that increasing moisture suppresses microorganisms or that decreasing moisture promotes microbial growth. The apparent inverse relationship is more plausibly explained by the structure of the experimental data: moisture content generally increased with storage time, whereas TPC, particularly in the nitrogen-based treatments, declined substantially during storage.

Consequently, both storage time and packaging treatment influenced the two variables simultaneously. The pooled Pearson coefficient therefore represents an overall statistical association across treatments and storage periods rather than a direct physiological effect of moisture content on microbial behavior. This distinction is particularly important in low-moisture foods because moisture

content and water activity ( $a_w$ ) represent different physicochemical properties. Moisture content describes the total amount of water present in the food matrix, whereas water activity more directly reflects the thermodynamic availability of water for microbial growth and survival [24]. Peleg demonstrated that microbial growth rate and growth initiation are strongly dependent on water activity, while recent reviews of low-moisture foods similarly emphasize the importance of  $a_w$  in determining microbial behavior [24], [37].

Microorganisms may remain viable for prolonged periods in low-water-activity foods even when conditions do not permit active growth. Their persistence is influenced by several interacting factors, including water activity, storage temperature, food-matrix characteristics, microorganism type, physiological state, and storage conditions [35], [38]. Igo and Schaffner showed that pathogen survival in low- $a_w$  foods is influenced by factors such as temperature, water activity, strain characteristics, and inoculation conditions, with substantial unexplained variation remaining among food matrices [32]. Liu et al. likewise reviewed the persistence and microbial-safety challenges associated with low-moisture foods and emphasized the importance of water activity and matrix-related factors [39]. Thus, the significant negative moisture-TPC correlation observed in this study should be interpreted as a statistical association within the experimental storage system rather than evidence of an inverse physiological dependence of microorganisms on water.

Ash content showed a positive correlation with  $\text{Log}_{10}$  TPC ( $r = 0.281$ ); however, the association was not statistically significant ( $P = 0.0616$ ). Therefore, the present data do not provide sufficient evidence for a significant bivariate relationship between ash content and microbial load. This result is biologically plausible because ash content represents the inorganic fraction of the sample and does not directly describe the water availability, gaseous environment, or other conditions required for microbial survival or proliferation. Accordingly, the positive coefficient should be considered only a weak statistical tendency within the present dataset and not evidence that higher mineral content promotes microbial activity. In contrast, moisture and ash contents showed a significant positive correlation ( $r = 0.663$ ,  $P < 0.001$ ), representing the strongest bivariate association among the variables evaluated.

This result indicates that variations in moisture and ash contents were not independent across treatments and storage periods. Because both variables are expressed relative to sample mass, changes in the relative proportions of other flour constituents may contribute to their covariation. However, protein, lipid, carbohydrate, and other proximate components were not measured longitudinally in the present study; therefore, the mechanism underlying this association cannot be conclusively determined from the available data.

Overall, the correlation analysis indicates that microbial stability in Beneng taro flour is multifactorial. Neither bulk moisture content nor ash content alone adequately explains variation in culturable microbial load. Microbial persistence in low-moisture foods can depend simultaneously on water activity, storage temperature, food-matrix structure and composition, microorganism characteristics, and other processing or environmental factors [32]. Accordingly, the Pearson coefficients observed in the present study should be interpreted as statistical associations specific to the experimental packaging and storage conditions rather than as direct causal relationships.

### 3.4 Multiple Linear Regression Analysis of the Association between Physicochemical Characteristics and Microbial Load

Multiple linear regression analysis was conducted to evaluate the simultaneous statistical associations of moisture content ( $X_1$ ) and ash content ( $X_2$ ) with microbial load expressed as  $\text{Log}_{10}$  TPC ( $Y$ ). The analysis included 45 paired observations obtained at weeks 0, 4, and 8. The fitted regression model was:

$$Y = 13.236 - 1.421X_1 + 2.319X_2$$

where Y represents  $\text{Log}_{10}$  TPC ( $\text{log}_{10}$  CFU/g),  $X_1$  represents moisture content (%), and  $X_2$  represents ash content (%).

**Table 5.** Multiple Linear Regression Analysis of Physicochemical Characteristics Associated with  $\text{Log}_{10}$  TPC of Beneng Taro Flour

| Variable                   | Regression coefficient (B) | Standard error | t-statistic | P-value               |
|----------------------------|----------------------------|----------------|-------------|-----------------------|
| Intercept                  | 13.236                     | 1.828          | 7.243       | $6.55 \times 10^{-9}$ |
| Moisture content ( $X_1$ ) | -1.421                     | 0.196          | -7.267      | $6.06 \times 10^{-9}$ |
| Ash content ( $X_2$ )      | 2.319                      | 0.334          | 6.953       | $1.70 \times 10^{-8}$ |

**Model statistics:**  $R^2 = 0.592$ ; adjusted  $R^2 = 0.573$ ;  $F(2,42) = 30.463$ ;  $P = 6.69 \times 10^{-9}$ ;  $n = 45$ .

The overall regression model was statistically significant ( $F(2,42) = 30.463$ ,  $P = 6.69 \times 10^{-9}$ ), indicating that moisture and ash contents were jointly associated with variation in microbial load under the experimental conditions. The coefficient of determination ( $R^2 = 0.592$ ) indicates that approximately 59.2% of the observed variation in  $\text{Log}_{10}$  TPC was statistically accounted for by moisture and ash contents included in the model, while approximately 40.8% remained unexplained. The adjusted  $R^2$  of 0.573 indicates a moderate explanatory capacity after accounting for the number of predictors. Moisture content showed a statistically significant negative regression coefficient ( $B = -1.421$ ,  $P = 6.06 \times 10^{-9}$ ).

Within the fitted model, a one-percentage-point increase in moisture content was associated with an estimated 1.421-unit decrease in predicted  $\text{Log}_{10}$  TPC when ash content was held constant. This coefficient should not be interpreted as evidence that increasing moisture content directly suppresses microbial survival. As demonstrated by the Pearson correlation analysis, the experimental observations pooled data across packaging treatments and storage periods; moisture content generally increased over time, whereas culturable microbial load declined markedly in some treatments. The negative coefficient therefore represents an adjusted statistical association within the experimental dataset rather than evidence of a direct inhibitory effect of moisture.

Ash content exhibited a statistically significant positive regression coefficient ( $B = 2.319$ ,  $P = 1.70 \times 10^{-8}$ ). With moisture content held constant, a one-percentage-point increase in ash content was associated with an estimated 2.319-unit increase in predicted  $\text{Log}_{10}$  TPC. This coefficient likewise should not be interpreted as evidence that greater mineral content directly stimulates microbial proliferation. Ash content represents the inorganic fraction remaining after combustion and is expressed relative to sample mass.

Therefore, variation in the relative proportions of moisture and other matrix constituents may influence the measured ash percentage. Because protein, lipid, carbohydrate, and other compositional fractions were not monitored longitudinally in the present study, the compositional basis of this association cannot be established from the available data. An important feature of the results is that ash content showed a weak and statistically non-significant bivariate correlation with  $\text{Log}_{10}$  TPC ( $r = 0.281$ ,  $P = 0.0616$ ), whereas its regression coefficient became statistically significant after adjustment for moisture content. This difference indicates that the estimated relationship between ash content and microbial load changed after moisture content was incorporated into the multivariable model.

This pattern suggests that the statistical association between ash content and  $\text{Log}_{10}$  TPC became apparent only after variation associated with moisture content was statistically controlled. Thus, the regression coefficient represents the partial association between ash content and  $\text{Log}_{10}$  TPC after statistically controlling for moisture content and should not be interpreted in the same manner as the simple Pearson correlation coefficient.

Moisture and ash contents were themselves moderately correlated ( $r = 0.663$ ), raising the need to consider potential multicollinearity. However, the variance inflation factor (VIF) was 1.786 for both predictors, indicating that multicollinearity was not substantial in the fitted model. Thus, both physicochemical variables could be retained simultaneously as predictors. Nevertheless, their coefficients should be interpreted cautiously because the regression was based on observations pooled across packaging treatments and storage periods.

Although the model accounted for 59.2% of the variation in  $\text{Log}_{10}$  TPC, approximately 40.8% remained unexplained. This residual variation is consistent with the multifactorial nature of microbial persistence in low-moisture food systems. Previous studies have demonstrated that microbial behavior in low-moisture foods can be influenced by water activity, storage temperature, microorganism type, food-matrix structure and composition, and other environmental or processing conditions [39].

Water availability is particularly important because total moisture content alone does not necessarily describe the state or availability of water within a food matrix; water activity and water mobility provide additional information relevant to microbial behavior [36]. Therefore, variables not incorporated into the present model particularly water activity, headspace gas composition, package relative humidity, package gas and water-vapor permeability, storage conditions, and food-matrix characteristics may account for part of the unexplained variation.

Furthermore, because packaging treatment and storage time were not included as predictors in the regression model, the estimated coefficients for moisture and ash contents may partly reflect treatment- and time-related variation. Therefore, the regression analysis should be interpreted as exploratory and explanatory rather than predictive or causal. The significant coefficients demonstrate associations between the measured physicochemical characteristics and culturable microbial load within the experimental system, but they do not establish that independently manipulating moisture or ash content would produce the predicted changes in TPC. Future studies incorporating water activity, headspace gas composition, package relative humidity, package barrier properties, and longitudinal proximate-composition measurements could improve both the explanatory capacity and mechanistic interpretation of the model.

#### 4. Conclusion

This study demonstrated that packaging treatment, storage duration, and their interaction significantly affected the culturable microbial load of Beneng taro flour during 12 weeks of storage. Among the treatments, the nitrogen-based treatments P2 (100%  $\text{N}_2$ ) and P4 (100%  $\text{N}_2$  + densified dry bacterial cellulose) showed the lowest TPC values at week 12, reaching  $263 \pm 116$  and  $387 \pm 131$  CFU/g, respectively. Moisture content was significantly affected by both packaging treatment and storage time, whereas ash content was significantly affected by packaging treatment but not by storage time. Correlation analysis showed a significant negative association between moisture content and  $\text{Log}_{10}$  TPC, while the association between ash content and  $\text{Log}_{10}$  TPC was not statistically significant.

Multiple linear regression further showed that moisture and ash contents jointly accounted for 59.2% of the observed variation in  $\text{Log}_{10}$  TPC. However, these relationships represent statistical associations within the experimental conditions and should not be interpreted as causal effects. Overall, the results indicate that nitrogen-based modified atmosphere packaging is a promising approach for maintaining the microbiological quality of Beneng taro flour during storage. Densified dry bacterial cellulose also showed potential as a complementary moisture-management component, as treatments containing bacterial cellulose exhibited comparatively lower moisture contents at week 8. However, its independent contribution and possible interaction with the modified atmosphere cannot be conclusively established from the present experimental design. Further studies incorporating water activity, package relative humidity, headspace gas composition, and gas and water-vapor barrier properties are recommended to clarify the mechanisms governing storage stability and to optimize the combined MAP bacterial cellulose packaging system.

## 5. Acknowledgement

This study was supported by the 2026 BIMA Prototype Assistance Grant, Directorate of Research and Development, Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, under Grant No. 2385/DST/PL.27/AL.04.03/2026.

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