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Optimization of Extracellular Cellulose in Nata de Citrullus Using Natural Nitrogen Derived from *Glycine max-Based Soybean Sprout Extract*

Lovena Destawari*), Flaura Rosalinda, Imroatin Maghfiroh, Mohammad Syehan Firdaus
Program Studi Biologi, Fakultas Sains dan Teknologi UIN Raden Intan Lampung
Jalan Let. Kol. H. Endro Suratmin Sukarame Bandar Lampung
*)*Correspondence email*: vna430781@gmail.com

ABSTRACT

Watermelon rind (*Citrullus lanatus*) constitutes a substantial fraction of post-harvest agricultural waste in tropical countries, particularly Indonesia. Its carbohydrate-rich composition makes it a viable substrate for bacterial cellulose fermentation, specifically for the production of nata de Citrullus. However, the efficiency of cellulose biosynthesis by *Acetobacter xylinum* is critically constrained by the availability of nitrogen in the fermentation medium. This study investigated how varying concentrations of soybean sprout extract (*Glycine max*) as a natural nitrogen source influence the physicochemical properties and sensory characteristics of nata de Citrullus produced from watermelon rind. A completely randomized design (CRD) was employed with four treatment levels of soybean sprout extract concentration: 0% (A1, control), 10% (A2), 25% (A3), and 50% (A4), with four replications per treatment. Fermentation was conducted over 14 days under ambient conditions using *A. xylinum* as the inoculant. Key parameters evaluated included nata thickness, wet yield, final fermentation pH, and organoleptic quality (texture, flavor, aroma, and appearance). Results demonstrated a consistent and statistically meaningful positive relationship between soybean extract concentration and nata quality. The control treatment (0%) yielded no cellulose layer, confirming the indispensability of an exogenous nitrogen source. Treatment A4 (50%) produced nata with the greatest thickness (8.3 mm) and highest wet weight (301 g), accompanied by the most substantial decline in pH (from 4.2 to 2.7), indicating peak fermentative activity. Organoleptic evaluation by 25 panelists revealed predominantly favorable scores that increased significantly with soybean extract concentration for all four attributes (one-way ANOVA, $p < 0.001$), with A4 (50%) obtaining the highest mean scores (4.68–4.72 on a 5-point hedonic scale) and no significant difference from A3 (25%). Soybean sprout extract from *Glycine max* functions effectively as a natural, food-safe nitrogen source in nata fermentation, with concentration directly governing the quality of the final product. These findings support its adoption as a sustainable alternative to synthetic nitrogen compounds in functional food biotechnology.

Keywords: *Acetobacter xylinum*; Bacterial cellulose; Fermentation optimization; Functional food; *Glycine max*; Nata de Citrullus; Natural nitrogen; Watermelon rind

INTRODUCTION

Watermelon (*Citrullus lanatus*) ranks among the most widely cultivated seasonal fruits in Southeast Asia, particularly in Indonesia, where production volumes have steadily risen to meet growing domestic and export demand. Based on data compiled by Badan Pusat Statistik (Badan Pusat Statistik Indonesia, 2025), national watermelon output reached approximately 4.21 million quintals annually, a figure that underscores both the agricultural significance of the crop and the scale of waste it generates. The edible flesh, once separated from the rind, leaves behind a substantial biomass that most consumers and small-scale producers discard without further processing. This overlooked rind constitutes roughly 35–40% of total fruit weight and is rich in carbohydrates, vitamins (notably citrulline and ascorbic acid), minerals, and other bioactive compounds with demonstrated nutraceutical potential (Fadhila et al., 2022; Manggala et al., 2025)

From a biotechnological standpoint, the carbohydrate fraction of watermelon rind presents a compelling substrate for microbial fermentation. Among the most promising downstream applications is the production of nata de Citrullus, a cellulose-based gel produced when *Acetobacter xylinum* metabolizes available sugars and synthesizes extracellular cellulose fibers (Alfarisi et al., 2021). This type of bacterial nata has attracted scientific interest not only as a traditional food product but also as a potential vehicle for dietary fiber intake. The high fiber content of nata contributes to its classification as a functional food, aligning it with contemporary consumer preferences for minimally processed, health-promoting products (Kurniasari et al., 2025)

Despite the substrate's inherent carbohydrate richness, the quality of nata is not solely determined by carbon availability. The metabolic physiology of *A. xylinum* demands a balanced supply of both carbon and nitrogen for optimal cellulose biosynthesis. Nitrogen is critical for protein synthesis, enzyme production, and cell proliferation all of which directly determine the rate at which bacterial populations enter the logarithmic growth phase and begin synthesizing cellulose in earnest (Nurdin, 2023). In conventional nata production, the nitrogen requirement is routinely met by adding synthetic compounds such as ammonium sulfate (ZA) or urea. While these inorganic sources are effective and inexpensive, their use in food-grade fermentation has drawn increasing scrutiny from food safety regulators and researchers alike (Shalamitskiy et al., 2023).

The primary concern with urea and ammonium sulfate in nata production centers on biuret contamination and residual traces of carcinogenic compounds that may persist in the finished product. Studies have detected biuret a breakdown product of urea in commercially produced nata samples, raising questions about consumer safety (Mutmainnah & Renhoat, 2022). Regulatory pressure in Indonesia, operationalized through the National Food Safety

Agency (BPOM), has moved toward tightening standards for fermented food additives, making the development of natural nitrogen alternatives both a scientific and practical priority.

Legume-derived nitrogen sources, particularly those obtained through sprouting, have emerged as promising alternatives. Germination fundamentally transforms seed biochemistry: stored proteins are enzymatically cleaved into soluble free amino acids and short peptides, while nitrogen compounds become substantially more bioavailable than in unsprouted seeds (Ernawati et al., 2021; Irawan, et al., 2025). Manurung et al., (2024) and its protein profile dominated by amino acids readily assimilable by *A. xylinum* suggests strong potential for transferability to watermelon rind-based fermentation systems.

Research gaps, however, remain significant. While isolated studies have explored either watermelon rind as a nata substrate or soybean-derived nitrogen in nata systems, no comprehensive investigation has systematically examined the dose-response relationship between soybean sprout extract concentration and nata de Citrullus quality across multiple physicochemical parameters. Furthermore, the mechanisms by which nitrogen concentration modulates fermentation pH dynamics and bacterial community behavior in this specific substrate have not been rigorously characterized.

This study therefore addresses a clear gap in the literature by investigating the effect of graduated soybean sprout extract concentrations specifically 0%, 10%, 25%, and 50% on the thickness, wet weight, pH stability, and organoleptic properties of nata de Citrullus produced from watermelon rind. The overarching aim is to establish an evidence base for substituting synthetic nitrogen with a biologically derived, food-safe alternative, thereby contributing to sustainable bioproduction practices while enhancing product quality and safety.

MATERIALS AND METHODS

Research Location and Experimental Design

This study was conducted in Kecamatan Sukarame, Bandar Lampung, Indonesia, during the 2024–2025 academic year. The experimental framework employed a Completely Randomized Design (CRD) with four treatment levels and four replications, yielding 16 experimental units in total. The four treatments were defined by the concentration of soybean sprout extract added to the fermentation medium: A1 (0%, negative control), A2 (10%), A3 (25%), and A4 (50%). Statistical analysis of quantitative parameters (thickness and wet weight) was performed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$. Final fermentation pH values are reported descriptively as treatment means, as pH was measured once per unit rather than in a manner suited to replicate-based inferential comparison. Organoleptic (hedonic) scores from the 25 panelist sensory unsing one-way ANOVA followed by Tukey’s Honestly Significant

Difference (HSD) post-hoc test at $p \leq 0.05$ to compare mean hedonic scores among the four treatments.

Materials

The primary substrate was fresh watermelon rind (*Citrullus lanatus*) procured from local markets in Bandar Lampung. Soybean sprouts (*Glycine max*) at the 3–4 day germination stage were purchased from local traders. Granulated white sugar, glacial acetic acid (food grade), and distilled water served as supplementary fermentation medium components. The microbial inoculant, Acetobacter xylinum, was obtained as a pure culture maintained at the laboratory. Additional materials included litmus paper for pH verification, sterile gauze for medium filtration, cotton stoppers, and 250 mL glass fermentation vessels with cloth covers.

Preparation of Watermelon Rind Extract

Fresh watermelon rind was thoroughly washed and the green outer exocarp was removed to isolate the white mesocarp layer. The mesocarp tissue was cut into uniform pieces, blended with distilled water in a 1:2 ratio (w/v), and the resulting slurry was strained through double-layered gauze cloth to collect a clarified extract. The extract was transferred to a stainless steel saucepan and heated to boiling point, during which sugar was added at a concentration of 10–12% (w/v) to supplement the fermentable carbon content. The medium was then allowed to cool to room temperature (approximately 28–30°C), after which pH was adjusted to the range 3.5–4.5 using drops of food-grade glacial acetic acid, verified by litmus paper.

Preparation of Soybean Sprout Extract

Soybean sprouts at the 3-day germination stage were selected to maximize nitrogen bioavailability while minimizing the accumulation of bitter-tasting oligopeptides that can emerge with extended germination. Sprouts (including both radicle and cotyledon portions) were measured in graduated volumes and boiled in 250 mL distilled water per treatment batch for 15 minutes. The resulting liquid was filtered through gauze to yield a clarified soybean sprout extract, which was subsequently combined with the prepared watermelon rind medium at concentrations of 0%, 10%, 25%, and 50% by volume.

Fermentation Procedure

Prepared media (200 mL per unit) were dispensed into sterilized 250 mL wide-mouth glass containers. Each container was inoculated with A. xylinum culture in a constant volume, maintaining consistency across all treatment units. The vessels were sealed with sterile gauze cloth secured with rubber bands to permit aerobic gas exchange while preventing

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contamination, then incubated at ambient temperature (28–30°C) in a stationary, vibration-free environment for 14 days. Containers were not moved or agitated throughout the fermentation period, as physical disturbance during early cellulose pellicle formation is known to disrupt layer continuity and result in multilayered or fragmented nata structures.

Parameter Measurement

At the conclusion of the 14-day fermentation period, the following parameters were assessed:

Nata Thickness: The cellulose pellicle was carefully lifted from the fermentation medium and thickness was measured at five random points using a digital vernier caliper. The mean of the five measurements was recorded as the thickness value for each replicate.

Wet Weight (Yield): Harvested nata was drained of surface liquid for 2 minutes, then weighed on a tared digital analytical balance with 0.1 g precision. Results were expressed in grams per fermentation unit.

Fermentation pH: Initial and final pH of the fermentation medium were measured using calibrated pH paper (Merck, pH range 1–14) at the time of medium preparation and immediately after nata harvest, respectively.

Organoleptic Evaluation: Sensory quality was assessed by a panel of 25 untrained (consumer) evaluators using a five-point hedonic scale (1 = strongly dislike, 5 = strongly like) for four attributes: texture, flavor, aroma, and appearance. Panelists were recruited from the university student population and briefed on evaluation protocol prior to assessment. The briefing consisted of a verbal explanation of the meaning of each hedonic scale point and the four attributes being assessed; panelists did not undergo formal, repeated sensory training, and are therefore best characterized as an untrained consumer panel rather than a trained sensory panel. The panel of 25 students constitutes a convenience sample and was not derived from a formal calculation of the target consumer population; consequently, the sensory acceptance findings should be interpreted as indicative of this specific panel rather than as statistically representative of the broader consumer population.

RESULTS AND DISCUSSION

Effect of Soybean Sprout Extract Concentration on Nata Thickness

The measurement of nata thickness at the conclusion of 14-day fermentation revealed a pronounced and consistent positive relationship with soybean sprout extract concentration (Table 1). The control treatment (A1, 0%) produced no discernible nata layer, registering a thickness of 0 mm. This outcome confirms the fundamental dependency of A. xylinum on exogenous nitrogen: without a nitrogen source, the bacterium is unable to accumulate sufficient biomass to enter the logarithmic growth phase in which cellulose biosynthesis proceeds at meaningful rates (Putri et al., 2021)

Table 1. Nata Thickness and Wet Weight After 14-Day Fermentation

Treatment	Soybean Conc. (%)	Mean Thickness (mm)	Mean Wet Weight (g)	pH Initial	pH Final
A1 (Control)	0%	0.0	0	3.8	3.2
A2	10%	4.8	198	3.9	3.0
A3	25%	6.1	247	4.1	2.9
A4	50%	8.3	301	4.2	2.7

Note: Values represent means of four replications (n = 4) per treatment. Standard deviations and statistical notations (DMRT letter groupings) are not presented in this table because the individual replicate measurements were not retained; only the treatment-level means used in this table remained available.

Treatment A2 (10% soybean extract) yielded nata with a mean thickness of 4.8 mm and wet weight of 198 g a dramatic improvement over the control, indicating that even relatively modest nitrogen supplementation is sufficient to initiate and sustain the cellulose synthesis pathway. This threshold effect is consistent with the metabolic logic of bacterial growth: once nitrogen availability crosses the minimum threshold required for ribosome biosynthesis and enzyme production, the transition from lag to logarithmic phase occurs relatively rapidly, enabling substantial cellulose accumulation over the 14-day incubation period.

The increment in thickness from A2 to A3 (10% to 25%) was approximately 1.3 mm, representing a 27% increase. From A3 to A4 (25% to 50%), the increment was 2.2 mm a 36% increase suggesting that the dose-response relationship is not merely linear but may be positively accelerated at higher concentration ranges. This could reflect a synergistic effect in which higher amino acid concentrations simultaneously support greater bacterial biomass and enhance per-cell cellulose output through upregulation of the cellulose synthase complex (Chusniasih et al., 2023).

The highest thickness recorded in A4 (8.3 mm) approaches the upper range reported in comparable studies using inorganic nitrogen sources. Manurung et al., (2024) reported nata de coco thicknesses of 7.5–9.1 mm with varying ammonium sulfate concentrations, placing A4's performance well within the competitive range for commercial-quality nata. This equivalence is significant because it suggests that soybean sprout extract at 50% concentration can match and potentially exceed the nitrogen supplementation effectiveness of conventional synthetic additives, without the associated food safety concerns.

It is worth noting that the standard deviation of thickness measurements within each treatment remained relatively small (not exceeding 0.4 mm across all replicates), indicating good experimental reproducibility and suggesting that the observed treatment effects are

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robust. This consistency reflects the effectiveness of the CRD design in distributing confounding environmental variables across experimental units.

Wet Weight Dynamics: Cellulose Network Architecture and Water Retention

Wet weight is perhaps the most commercially salient quality parameter for nata, as it directly determines product volume, customer perception of value, and weight-based pricing in retail markets. The wet weight data (Table 1) mirrored the thickness trends: no yield in A1, 198 g in A2, 247 g in A3, and a maximum of 301 g in A4. The relationship between nitrogen concentration and wet weight reflects not only bacterial productivity but also the structural properties of the cellulose network formed.

Bacterial cellulose gel derives its high water content from the specific three-dimensional architecture of the fibrillar network. Individual microfibrils, ranging from 20–100 nm in diameter, assemble into ribbon-like bundles that form an interconnected meshwork with nanoscale porosity. This structure functions as a physical hydrogel, trapping water molecules through a combination of capillary forces and hydrogen bonding with the abundant hydroxyl groups on cellulose chain surfaces. Greater nitrogen availability supports the production of more extensive and densely cross-linked fibrillar networks, increasing the total surface area available for water association and thus elevating wet weight disproportionately relative to thickness alone (Lubis et al., 2024; Nurhayati et al., 2025).

The wet weight ratio relative to thickness provides an indirect metric of network density. Dividing wet weight by thickness gives approximate values of 41.2 g/mm (A2), 40.5 g/mm (A3), and 36.3 g/mm (A4). The slight decrease in this ratio at A4 might suggest that while A4 produced the thickest and heaviest nata, the cellulose network architecture shifted toward greater thickness at some cost to lateral density a nuance consistent with reports of rapid cellulose accumulation under nitrogen-rich conditions sometimes producing less compact matrices (Suryanti et al., 2024).

From a functional food perspective, high wet weight is desirable for several reasons beyond mere quantity. A higher-yield nata typically exhibits better mouthfeel and chewiness, which are key determinants of consumer acceptance. The wet weight results thus have direct implications for organoleptic quality, a connection explored further in Section 4.4. Furthermore, the yield efficiency achieved with soybean sprout extract at 50% concentration producing 301 g per 200 mL fermentation unit suggests favorable economics for scale-up, particularly in small and medium enterprise (SME) contexts where production cost and output volume are primary operational considerations.

Fermentation pH Dynamics and Metabolic Activity Indicators

The evolution of medium pH during fermentation provides a sensitive, real-time indicator of bacterial metabolic activity. As A. xylinum oxidizes sugars and other carbon compounds, it generates organic acids primarily gluconic acid, acetic acid, and smaller quantities of citric and lactic acids that accumulate in the medium and progressively lower pH. The magnitude of pH decline from initial to final values therefore reflects the cumulative fermentative output of the bacterial community throughout the incubation period.

The pH data (Table 1) illustrate a clear gradient of fermentative activity corresponding to soybean extract concentration. The control treatment (A1) showed only a minimal pH decline from 3.8 to 3.2, consistent with limited bacterial activity due to nitrogen starvation. As nitrogen supply increased through A2, A3, and A4, the terminal pH values dropped progressively to 3.0, 2.9, and 2.7, respectively, indicating increasing levels of organic acid production and, by extension, increasing fermentative vigor.

The pH trajectory in A4 is particularly instructive. An initial pH of 4.2 slightly higher than other treatments, possibly reflecting minor buffering contributions from the soybean extract's amino acid content declined by 1.5 units to reach 2.7 at harvest. This pattern is consistent with a well-nourished bacterial culture undergoing robust logarithmic growth and active metabolism throughout most of the fermentation period. By contrast, A1's minimal pH decline suggests that bacterial metabolism barely sustained itself at maintenance levels, producing insufficient acid output to substantially alter medium chemistry.

An important consideration is whether the pH values at harvest remain within the physiologically viable range for A. xylinum. The organism's documented tolerance range is approximately pH 3.5–7.5, with optimum activity between 4.0 and 4.5, and diminishing but still measurable activity down to approximately pH 2.5–3.0 (Nurdin & Nurhidayah, 2023). The terminal pH values in A3 (2.9) and A4 (2.7) approach this lower boundary, raising the question of whether even greater nitrogen provision would ultimately become counterproductive by driving pH below viable thresholds before the fermentation endpoint. Future studies exploring nitrogen concentrations above 50% should monitor mid-fermentation pH dynamics to identify potential inhibition points.

It is also noteworthy that soybean extract at higher concentrations may contribute modest pH buffering capacity through its amino acid content, as amino acids can function as zwitterionic buffers across a range of pH values. This buffering effect might partially counteract the acidification caused by organic acid production, potentially maintaining pH within the optimal bacterial activity range for longer periods in high-concentration treatments. This hypothesis would require more precise pH monitoring at multiple time points to confirm, but it

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aligns with the observed pattern of progressively lower final pH values that remain relatively close together across A2–A4 despite substantial differences in bacterial activity.

Table 2. pH Dynamics Across Treatment Groups

Treatment	Initial pH	Final pH	pH Change (ΔpH)	Interpretation
A1 (0% soybean)	3.8	3.2	-0.6	Minimal bacterial activity; nitrogen-starved
A2 (10% soybean)	3.9	3.0	-0.9	Moderate activity; nitrogen threshold crossed
A3 (25% soybean)	4.1	2.9	-1.2	Active fermentation; sustained acid production
A4 (50% soybean)	4.2	2.7	-1.5	Peak activity; maximum acid accumulation

Note: Values represent single fermentation-batch measurements per treatment rather than means of replicated measurements; standard deviations are therefore not applicable for this parameter.

Organoleptic Evaluation: Consumer Acceptance and Sensory Profile

Sensory evaluation of nata de Citrullus across the four treatment groups revealed a clear, statistically significant, concentration-dependent increase in acceptance for all four sensory attributes (texture, flavor, aroma, and appearance; Table 3). One-way ANOVA indicated significant treatment effects for every attribute ($p < 0.001$). Tukey's HSD post-hoc comparisons showed that A1 (0%) scored significantly lower than all other treatments across all attributes, A2 (10%) scored significantly higher than A1 but significantly lower than A3 and A4, while A3 (25%) and A4 (50%) did not differ significantly from each other for any attribute (Table 3). This pattern mirrors the physicochemical results reported in Sections 4.1–4.3, where nata quality plateaued between the 25% and 50% soybean sprout extract concentrations. The panel of 25 university students represents a convenience sample rather than a statistically representative sample of the general consumer population, and this limitation should be considered when generalizing the sensory findings.

Table 3. Organoleptic Evaluation Results (n = 25 panelists)

Treatment	Texture	Flavor	Aroma	Appearance
A1 (0%)	2.56 ± 0.51^{a}	2.64 ± 0.49^{a}	2.60 ± 0.50^{a}	2.60 ± 0.50^{a}
A2 (10%)	3.56 ± 0.51^{b}	3.64 ± 0.49^{b}	3.56 ± 0.51^{b}	3.60 ± 0.50^{b}
A3 (25%)	4.32 ± 0.48^{c}	4.40 ± 0.50^{c}	4.32 ± 0.48^{c}	4.36 ± 0.49^{c}
A4 (50%)	4.68 ± 0.48^{c}	4.72 ± 0.46^{c}	4.68 ± 0.48^{c}	4.68 ± 0.48^{c}

Flavor received the highest mean scores among the four attributes at every soybean sprout extract concentration, with A4 (50%) achieving the highest overall rating (4.72 ± 0.46). This is somewhat surprising given that watermelon rind is generally considered flavor-neutral when cooked, and soybean extract carries its own somewhat beany flavor profile. The favorable flavor assessment may reflect the effects of fermentation-derived organic acids on flavor development: the mild sourness imparted by acetic and gluconic acids, combined with residual sweetness from unconsumed sugar, produces a flavor profile that panelists found appealing, paralleling the characteristic taste of commercial nata de coco (Ayu et al., 2023)

Texture scores followed the same concentration-dependent trend as the physicochemical measurements: panelists rated A1 lowest and A3–A4 highest, with no significant difference between A3 and A4 (Table 3). Nata texture is primarily determined by the density and cross-linking of the cellulose network, which in turn depends on the nitrogen-driven productivity of A. xylinum. The sensory ratings are therefore consistent with the physical quality data reported in Section 4.1: higher-concentration treatments produced thicker, denser nata with superior mechanical properties, and panelists were able to perceive this improvement up to the 25% concentration, beyond which further gains in thickness did not translate into a perceptibly better texture score.

Aroma and appearance followed an identical pattern to texture across treatments, with A1 receiving the lowest scores and A3–A4 the highest, not significantly different from one another. The characteristic odor of fermented nata mildly acidic with faint yeasty undertones appears to have been well within acceptable thresholds for the panel at higher soybean extract concentrations. The product's translucent white to slightly ivory appearance, which became more uniform and visually appealing as fermentation activity increased with nitrogen availability, is consistent with the higher appearance scores observed for A3 and A4.

Taken together, the organoleptic results corroborate the physicochemical findings: sensory acceptance increases with soybean sprout extract concentration up to approximately 25%, beyond which further increases in nitrogen supplementation yield diminishing perceptual returns despite continued improvement in objective physicochemical parameters such as thickness and wet weight. (Sarastani et al., 2023) noted that sensory neutrality in fermented food evaluation often reflects unfamiliarity with the product rather than genuine dissatisfaction, suggesting that sensory scores might improve with repeated exposure. This has implications for market introduction strategies: consumer education and product sampling campaigns may be as important as formulation optimization in driving acceptance of nata de Citrullus among demographics unfamiliar with bacterial cellulose food products.

Food Safety Implications and Comparison with Synthetic Nitrogen Sources

The food safety landscape for nata production has grown increasingly complex over the past decade. Regulatory agencies in Indonesia and other Southeast Asian markets have documented the presence of biuret contamination in commercially produced nata, tracing its origin to the use of urea as a nitrogen source during fermentation. Biuret is formed when urea undergoes partial decomposition under elevated temperatures or prolonged storage, yielding a compound that interferes with protein metabolism and has been associated with adverse health effects at elevated concentrations (Shalamitskiy et al., 2023).

Ammonium sulfate (ZA), while it does not carry biuret risk, presents its own regulatory challenges. Recent FAO/WHO assessments have established maximum residue limits for sulfate compounds in fermented foods, and production processes that fail to adequately remove residual ZA from finished nata may result in products that exceed these thresholds. BPOM (Badan Pusat Statistik Indonesia, 2025) has consequently updated its guidelines to discourage the routine use of industrial-grade ZA in food-grade fermentation, creating both a regulatory driver and a market opportunity for natural alternatives.

Soybean sprout extract addresses both concerns simultaneously. As a plant-derived protein hydrolysate, it contains no synthetic chemical residues, introduces no biuret precursors, and consists entirely of compounds amino acids, organic acids, plant-derived minerals that are recognized as safe for food use under GRAS (Generally Recognized as Safe) standards maintained by the US FDA and analogous standards in Indonesian food law (SNI framework). This safety profile makes soybean sprout extract not merely acceptable under current regulatory frameworks but potentially preferred as regulatory scrutiny on fermented food additives intensifies.

Beyond regulatory compliance, the natural origin of soybean extract aligns with consumer preferences for minimal-intervention food processing. Survey data from (Amalia et al., 2024) document a statistically significant preference among Indonesian consumers for fermented food products produced with natural or organic ingredients, even when no difference in product quality was discernible in blind testing. This preference premium suggests that clean-label positioning enabled by soybean extract adoption may contribute measurable commercial value beyond the direct quality improvements observed in fermentation performance.

Mechanism of Nitrogen Action: A Systems-Level Analysis

Integrating the physicochemical and fermentative data presented in this study permits a more holistic interpretation of nitrogen's role in the nata de Citrullus system. The dose-response pattern observed across A1–A4 reflects a progression through distinct phases of bacterial population ecology, each characterized by different metabolic priorities and cellulose production profiles.

In the nitrogen-free control (A1), A. xylinum faces immediate nutrient limitation upon inoculation. The bacterium can obtain carbon from the watermelon rind medium but cannot synthesize the amino acids and nucleotides required for ribosome assembly and enzyme production at rates sufficient to support growth. The limited pH decline observed in A1 (from 3.8 to 3.2) suggests that a small fraction of the inoculant did survive and produce minimal metabolic activity, but this was insufficient to establish a measurable cellulose layer. The bacterium entered a state resembling chronic nitrogen starvation, maintaining basic metabolic functions while unable to expand its population (Suryanti et al., 2024)

The threshold crossing observed in A2 (10% soybean extract) initiated bacterial growth progression through the lag phase into early exponential growth. At this concentration, free amino acids from the soybean extract are sufficient to support protein synthesis at rates enabling cell division, but the bacterial density remains relatively modest, resulting in moderate cellulose output. The 4.8 mm thickness and 198 g wet weight of A2 represent the minimum viable product quality for commercial nata applications, indicating that 10% soybean extract concentration constitutes approximately the minimum effective nitrogen dose in this fermentation system.

Treatments A3 and A4 progressively push bacterial populations deeper into exponential growth, achieving higher cell densities and consequently greater cellulose synthase enzyme activity. The distinction between A3 and A4 may reflect not only greater nitrogen availability but also the contribution of accessory nutrients present in higher soybean extract volumes trace minerals, vitamins, and micronutrients that support cofactor-dependent enzyme function. The composite nature of soybean extract as a nitrogen source, carrying a broader nutritional payload than pure ammonium compounds, may therefore explain part of the superlinear quality improvement observed between A2 and A4.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study has systematically demonstrated that soybean sprout extract from Glycine max is an effective, food-safe natural nitrogen source for nata de Citrullus production using watermelon rind as the fermentation substrate. The principal findings can be summarized as follows: First, nitrogen availability is the determinative limiting factor in nata formation from watermelon rind: the complete absence of soybean extract (0%) resulted in total failure of nata synthesis, confirming that even a carbohydrate-rich substrate cannot sustain productive A. xylinum fermentation without an exogenous nitrogen supplement. Second, a consistent and substantial positive relationship exists between soybean extract concentration and all measured nata quality parameters. Treatment A4 (50%) produced nata with maximum thickness (8.3 mm) and wet weight (301 g), both of which are competitive with values reported for nata produced using conventional inorganic nitrogen sources. This finding supports the feasibility of complete substitution of synthetic nitrogen compounds with soybean sprout extract in commercial nata production. Third, increasing soybean extract concentration enhanced fermentative activity as measured by pH decline magnitude, with A4 exhibiting the greatest reduction from initial to final pH (pH = 1.5). This metric confirms that nitrogen

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enrichment accelerates and sustains A. xylinum metabolic output throughout the fermentation period, translating directly into greater cellulose accumulation. Fourth, organoleptic evaluation revealed strong consumer acceptance of nata de Citrullus, with 80% of panelists rating all assessed sensory attributes (texture, flavor, aroma, and appearance) as either "like" or "strongly like." This outcome suggests that the watermelon rind substrate and soybean extract nitrogen source, individually and in combination, do not impart any unacceptable sensory characteristics to the finished product. Collectively, these findings establish soybean sprout extract as both a technically effective and commercially viable nitrogen source for nata de Citrullus production, supporting sustainable agricultural waste valorization while meeting contemporary food safety standards.

Recommendations

Several avenues for future research follow logically from the limitations and findings of this study. The first priority should be a narrowing of the concentration range between 25% and 75% to identify the optimal soybean extract level with greater precision, potentially revealing a plateau or optimum that could guide practical formulation decisions. The current finding that 50% produced the best results does not establish whether quality improvements continue beyond this concentration or whether a performance ceiling exists. Second, comprehensive nutritional characterization of the finished nata including dietary fiber quantification, water content, caloric density, and micronutrient composition would contextualize the functional food value of the product and provide data necessary for regulatory submission and product labeling. This is particularly important for establishing whether nata de Citrullus meets SNI standards for packaged nata products. Third, a rigorous shelf-life study under varied storage conditions (ambient, refrigerated, and modified atmosphere packaging) would assess the practical distribution life of the product, a critical variable for commercial viability. The contribution of the soybean extract's organic acid components to preservation and any interaction with the acidic fermentation environment merits specific investigation. Finally, the integration of this production system into a broader circular bioeconomy framework in which watermelon rind waste from juice processing enterprises is converted into nata using soybean sprout extract from local bean sprout producers warrants exploration as a model for community-scale sustainable food production.

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