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Optimization of Crude Fiber Content in Nata Produced from Watermelon Rind through Variations in Molasses and Mung Bean Sprout Broth

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Abstract: Nata is a fermented product made from cellulose and synthesized by *Acetobacter xylinum*, offering potential as a functional food rich in dietary fiber. This study aimed to evaluate the effect of varying volumes of molasses and mung bean sprout (*Phaseolus radiatus* L.) broth as sources of carbon and nitrogen on the crude fiber content of nata produced from watermelon (*Citrullus lanatus*) rind waste. Each fermentation medium had a total volume of 1000 mL and included the following treatments: N1 (5% molasses and 5% mung bean sprout broth), N2 (10% molasses and 10% mung bean sprout broth), N3 (15% molasses and 15% mung bean sprout broth), and N0 (control), which used coconut water, granulated sugar, and food grade urea. Fermentation was carried out under static conditions at room temperature for 10 days, and the resulting nata was analyzed for crude fiber content using the gravimetric method. The results showed that variations in the volumes of molasses and mung bean sprout broth significantly influenced the crude fiber content of nata ($p < 0.05$). The DMRT further confirmed statistically significant differences among treatments. Treatment N2 produced the highest crude fiber content (8.05 ± 0.02%), while N0 yielded the lowest value (5.11 ± 0.01%). The absence of nata formation in N3 indicated substrate saturation and nutritional imbalance. These findings emphasize the importance of carbon and nitrogen balance in optimizing nata production through organic waste utilization. Treatment N2 was identified as the most effective in enhancing both crude fiber content and overall nata quality, with potential implications for functional food development, sustainable waste management, and contextual learning in school chemistry.

Keywords: Crude Fiber Content; Molasses; Mung Bean Sprout Broth; Watermelon Rind.

Introduction

The production of watermelon in East Java Province reached 107.902 tons in 2023 [1]. This high production volume results in a considerable amount of watermelon rind waste. Although biodegradable, watermelon rind is an organic waste that, if not managed properly, can have adverse effects on the environment. The accumulation of such waste can cause environmental issues and serve as a breeding ground for pathogenic microorganisms that may threaten public health [2]. Therefore, innovative and sustainable strategies for waste utilization are required to mitigate environmental impacts.

One promising solution is the conversion of organic waste into products with added value such as nata. Using watermelon rind as a substrate in nata production not only reduces waste but also provides economic benefits by generating a food product rich in dietary fiber. This approach aligns with the objectives of the Sustainable Development Goals, particularly those related to food security, waste management, and nutritional improvement. Watermelon rind contains organic compounds such as sugars, which serve as potential fermentation substrates for *Acetobacter xylinum* in nata production [3].

Nata is a fermented product composed of cellulose and produced by *Acetobacter xylinum* in a medium containing balanced sources of carbon and nitrogen [4]. During fermentation, carbon serves as an energy source, while nitrogen supports the synthesis of proteins and enzymes essential for microbial metabolism [5]. Although sucrose from granulated sugar is commonly used as the carbon source in nata production, molasses presents a viable alternative. Molasses contains approximately 35% sucrose, 7% glucose, 9% fructose, and other carbohydrates that can be utilized in fermentation processes [6]. In addition, mung bean sprout broth contains high nitrogen levels, ranging from 20-35%, making it a potential substitute for conventional nitrogen sources [7].

The formulation of a fermentation medium combining molasses and mung bean sprout broth may improve the efficiency of nata production, particularly in terms of increasing crude fiber content. Crude fiber in nata originates from microbial cellulose synthesized during fermentation [8]. As a component of dietary fiber, it may support digestive function and enhance the functional properties of nata as a nutritious food product.

Several previous studies have explored the potential use of watermelon rind in nata production and have shown favorable physical properties of the resulting product.

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Although various studies have utilized alternative substrates such as watermelon rind in nata production [9], few have specifically focused on optimizing crude fiber content. Other investigations have assessed molasses and mung bean sprout broth individually as carbon and nitrogen sources, respectively, in nata production [10][11]. Nonetheless, the potential synergy between the two has not been fully explored within a fermentation system based on watermelon rind extract.

This study aims to address that research gap and contribute to the development of environmentally responsible, biotechnology-driven functional food products. To date, no studies have reported the use of a combined formulation of molasses and mung bean sprout broth specifically for optimizing the crude fiber content in nata derived from watermelon rind. This combination represents a novel approach to enhance the nutritional value of nata using a more sustainable and organic waste-based fermentation strategy.

Additionally, the outcomes of this study can serve as a contextual reference for chemistry education, particularly within project-based learning models aligned with the Kurikulum Merdeka. By integrating waste management, microbial biotechnology, and nutritional analysis, this study highlights the interdisciplinary value of applied science in both academic and industrial contexts.

Research Methods

Research Design

This study employed a quantitative approach using an experimental method conducted in a laboratory setting. The experimental design used was a Completely Randomized Design (CRD) with a single treatment factor, which was the variation in the composition of the fermentation medium. The treatments included four groups: N0 as the control using a coconut water-based medium, and N1, N2, and N3 using fermentation media formulated from watermelon rind extract combined with molasses and mung bean sprout broth. All treatments were prepared using a total medium volume of 1000 mL and were carried out under controlled environmental conditions.

The use of CRD was based on the assumption that environmental conditions across all experimental units were homogeneous, and randomization was applied to minimize the risk of treatment bias. Each treatment was replicated six times (n = 6) to ensure the accuracy, consistency, and statistical validity of the results.

Tools and Materials

The tools used in this study included a blender, gauze, stainless steel pan, sterile plastic trays, baking paper, rubber bands, measuring cylinders, volumetric pipettes, metal clamps, 750 mL Erlenmeyer flasks, filter papers, an electric stove, an oven set at 105°C, an analytical balance with a precision of 0.0001 g, and porcelain crucibles.

The primary materials used in the nata fermentation process were watermelon rind (albedo), molasses, mung bean sprout (*Phaseolus radiatus* L.) broth, glacial acetic acid, and a *Acetobacter xylinum* starter culture. For the crude fiber analysis, the following reagents were utilized: 1.25% sulfuric acid (H_2SO_4), 3.25% sodium hydroxide (NaOH), 10% potassium sulfate (K_2SO_4), boiling distilled water, and 95% alcohol.

Research Variables

The independent variable in this study was the composition of the fermentation media, which involved varying concentrations of molasses as the carbon source and mung bean sprout broth as the nitrogen source. These components were simultaneously varied in three treatments: 5% (N1), 10% (N2), and 15% (N3) of the total fermentation media volume of 1000 mL.

The dependent variable was the crude fiber content of the nata produced through fermentation. This fiber content represents the cellulose synthesis activity by *Acetobacter xylinum*, which is influenced by the nutritional balance, particularly the carbon and nitrogen content in the fermentation media.

In addition to the three treatments, a control group (N0) was included, in which nata was fermented in 1000 mL of coconut water with the addition of 30 grams of granulated sugar and 1 gram of food-grade urea. This control served as a reference to evaluate the effectiveness of the fermentation medium formulated from watermelon rind extract in producing nata with optimal crude fiber content.

The composition of each fermentation medium for the four treatments is presented in Table 1.

Table 1. Composition of Fermentation Media for Each Treatment.

Treatment	Watermelon Rind Extract (mL)	Molasses (mL)	Mung Bean Sprout Broth (mL)
N0	-	-	-
N1	900	50	50
N2	800	100	100
N3	700	150	150

Nata Production Procedure

The nata production process began with the extraction of watermelon rind juice. A total of 1500 grams of albedo, which is the white inner layer of the rind, was separated and blended with 3 liters of water at a 1:2 ratio until a homogeneous mixture was obtained. The mixture was filtered using gauze to produce a clean extract free from solid particles.

The fermentation media were prepared by heating the watermelon rind extract according to each treatment formulation. Once the extract reached boiling temperature, molasses and mung bean sprout broth were added and stirred until homogeneous. The media were reheated to boiling temperature to ensure sterilization. After sterilization, the solution was poured into sterile trays and allowed to cool to room temperature.

When the media had cooled, 10 mL of glacial acetic acid were added to optimize the growth conditions for *Acetobacter xylinum*. This was followed by the inoculation of 100 mL of *Acetobacter xylinum* starter culture into the media. The fermentation trays were then covered with baking paper and secured with rubber bands to prevent contamination [12]. Fermentation was carried out at room temperature (27-30℃) for 10 days until a nata layer had formed.

On day 10, the nata was harvested by collecting the cellulose layer that had formed on the surface of the media [13]. The nata was then washed thoroughly to remove any remaining fermentation residue before proceeding to the crude fiber analysis.

This procedure utilized organic materials such as watermelon rind extract, molasses, and mung bean sprout broth as fermentation substrates. These biodegradable components support microbial growth while exemplifying the repurposing of food waste in biotechnology. The approach promotes sustainable practices and reflects ethical considerations by minimizing reliance on refined inputs and encouraging resource efficiency.

Crude Fiber Content Test

Crude fiber analysis was carried out based on gravimetric principles using a previously adapted method [14]. A total of 2 grams of nata sample were weighed and placed into a 750 mL Erlenmeyer flask, followed by the addition of 200 mL of 1.25% sulfuric acid solution. The flask was equipped with a vertical condenser and boiled for 30 minutes. After cooling, the mixture was filtered, and the residue was thoroughly rinsed with boiling distilled water until clean.

The residue was then transferred back into the Erlenmeyer flask and treated with 200 mL of 1.25% sodium hydroxide solution. The mixture was boiled again for 30 minutes. Afterwards, it was filtered once more. During this filtration step, the residue was washed sequentially with 10% potassium sulfate solution, boiling distilled water, and 15 mL of 95% ethanol.

The residue was then dried in an oven at 105 °C for 1 hour. Crude fiber content was determined based on the difference in mass before and after treatment.

Data Analysis

The data were analyzed using one-way Analysis of Variance (ANOVA) to evaluate the effect of varying volumes of molasses and mung bean sprout broth at 5%, 10%, 15% on the crude fiber content of nata. When a significant difference was observed ($p < 0.05$), the analysis was followed by Duncan's Multiple Range Test (DMRT) to identify specific differences among the treatment groups. DMRT was selected because it is a liberal, step-down procedure that conducts pairwise comparisons and is more sensitive in detecting subtle differences among treatment means, particularly when the number of groups is limited [15]. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 16.

Results and Discussion

Nata Formation Metabolism

Watermelon rind extract was used as the raw material in the fermentation process due to its high water content, which provides a suitable medium to support the growth of *Acetobacter xylinum*. This microorganism grows optimally in substrates containing essential nutrients such as water, protein, fat, carbohydrates, ash, and minerals [16]. To meet these nutritional requirements, molasses was added as a carbon source, while mung bean sprout broth served as a nitrogen source.

The nata formation process by *Acetobacter xylinum* involves three main biochemical stages: carbon source utilization, cellulose synthesis, and the aggregation and formation of the cellulose layer [17]. In the initial stage, the enzyme invertase hydrolyzes sucrose from molasses into glucose and fructose. These monosaccharides enter the glycolysis pathway and produce pyruvate along with energy in the form of ATP [18].

The resulting pyruvate is then converted into acetyl-CoA and enters the Krebs cycle. This process generates NADH and $FADH_2$, which serve as energy sources necessary for supporting bacterial cellular metabolism [19]. This energy drives the synthesis and secretion of cellulose to the surface of the fermentation medium.

The synthesized cellulose subsequently accumulates on the surface of the medium, forming a gel like structure or a three-dimensional cellulose matrix known as nata. This cellulose consists of glucose polymers linked by β-1,4 glycosidic bonds and exhibits a regular hydrogen bonding pattern. The structure forms micelles that assemble into cellulose fibrils. Its linear configuration and high crystallinity make it insoluble in water and resistant to enzymatic degradation [20].

Additionally, nitrogen availability in the fermentation medium plays a critical role in the cellulose synthesis process. Nitrogen supports the formation of proteins and enzymes required by *Acetobacter xylinum*, thus contributing to the formation of nata with dense, strong, and stable characteristics [11].

Effect of Treatment Variations

Based on the research results, not all treatments were able to form nata optimally. Treatments N0 (control), N1 (5%), and N2 (10%) showed successful formation of nata layers, whereas treatment N3 (15%) did not result in nata formation. Physical differences in color were observed among the formed nata. Nata from treatments N1 and N2 exhibited a brownish hue, with a darker intensity in treatment N2. This color variation was attributed to the higher volume of molasses used in the fermentation medium. Molasses contains natural pigment compounds derived from sugar industry byproducts that can accumulate in the nata matrix [10]. The visual differences in nata resulting from each treatment are illustrated in Figure 1.

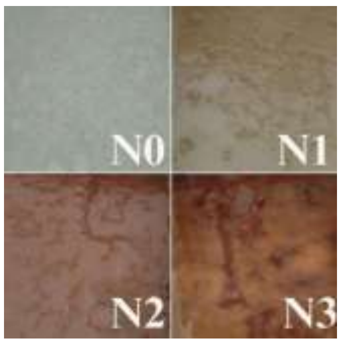

Figure 1. Nata Formation Outcomes Under Each Treatment Condition.

The successful nata formation in treatments N1 and N2 indicates that the addition of 5% and 10% molasses and mung bean sprout broth, respectively, from a total volume of 1000 mL of fermentation medium, was sufficient to supply the nutrients needed to support the metabolism of *Acetobacter xylinum*. This suggests that both the carbon and nitrogen sources were available in appropriate concentrations, allowing optimal bacterial growth and cellulose biosynthesis. The observed cellulose synthesis activity suggests that the carbon and nitrogen ratio in the medium remained within the optimal range to support enzymatic activity and bacterial growth. This finding is consistent with previous studies showing that a balanced carbon and nitrogen ratio is crucial for efficient microbial cellulose production [21].

In contrast, treatment N3, which used 150 mL of molasses and 150 mL of mung bean sprout broth in a 1000 mL medium, failed to form nata. This failure is most likely due to nutrient oversaturation, which disrupted the metabolism of *Acetobacter xylinum*. An excessively high concentration of both carbon and nitrogen can interfere with metabolic pathways essential for cellulose biosynthesis.

A higher total sugar concentration in the fermentation medium could lead to increased osmotic pressure and microbial stress, especially in the early stages of fermentation [22]. A high volume of molasses may result in the accumulation of unutilized sugars, which can lead to increased production of acetic acid. In excessive amounts, acetic acid is toxic to *Acetobacter xylinum*, inhibiting enzyme activity and reducing cellulose synthesis [23]. Additionally, excess nitrogen from mung bean sprout broth may worsen the nutrient imbalance. Based on studies in plant nitrogen metabolism, excessive nitrogen levels may disrupt ammonium assimilation and cellular balance, which could potentially inhibit microbial activity [24]. Unassimilated nitrogen may also contribute to metabolic stress, which can affect the structural formation of nata [25]. When the carbon, nitrogen, and inoculum ratios are optimally adjusted under controlled fermentation conditions, most of the liquid medium can be efficiently converted into nata without leaving significant residue [12].

Crude Fiber Content of Nata

The results of the one-way ANOVA test showed that variations in the volume of molasses and mung bean sprout broth in the fermentation medium had a significant effect on the crude fiber content of the resulting nata, with a p-value less than 0.05. This indicates that changes in the volumes of both ingredients significantly influenced cellulose synthesis by *Acetobacter xylinum* during fermentation.

Further analysis using Duncan's Multiple Range Test (DMRT) revealed that each treatment produced significantly different crude fiber levels. As the volumes of molasses and mung bean sprout broth increased, the crude fiber content of the nata also increased. The crude fiber values for each treatment are shown in Table 2.

These results suggest that the addition of molasses and mung bean sprout broth had a positive influence on the cellulose production of *Acetobacter xylinum*, directly contributing to the increase in crude fiber content of the nata. Molasses served as a primary carbon source, enhancing the polymerization of glucose into β-1,4-glucan chains essential for cellulose biosynthesis [26]. Meanwhile, the mung bean sprout broth provided a nitrogen source that supported bacterial metabolism, thereby promoting a higher cellulose yield [27].

Table 2. Comparison of Crude Fiber Content.

Treatment	Crude Fiber Content (%)
N0	5.11 ± 0.01^{b}
N1	7.18 ± 0.03^{c}
N2	8.05 ± 0.02^{d}

Note: Treatments with the same superscript letters indicate no significant difference, while different superscripts indicate a significant difference at α = 0.05.

The control treatment (N0) had the lowest crude fiber content at 5.11 ± 0.01%, using a fermentation medium based on coconut water, granulated sugar, and food-grade urea. Treatment N1, with a 5% volume, produced a crude fiber content of 7.18 ± 0.03%, while treatment N2, with a 10% volume, yielded the highest value at 8.05 ± 0.02%.

The increase in crude fiber content is strongly associated with the availability of sucrose in molasses, which is enzymatically hydrolyzed into glucose and fructose, then utilized as substrates in the cellulose biosynthetic pathway [28]. High carbon availability promotes robust polysaccharide synthesis, while sufficient nitrogen enhances bacterial growth and enzymatic activity in *Acetobacter xylinum*.

In treatment N2, the elevated yet balanced carbon and nitrogen ratio supported bacterial growth and increased cellulose production. This aligns with previous studies showing that appropriate supplementation of nitrogen can significantly enhance crude fiber levels in nata fermentation [29].

These results are in line with previous research indicating that fruit based substrates such as pineapple liquid waste, when supplemented with additional nutrients, significantly increased the crude fiber content of the resulting nata, with the highest value reaching 9.3% [30]. Just as in the present study, nutrient dense substrates were effective in improving bacterial activity and cellulose output, emphasizing the pivotal role of a balanced carbon and nitrogen ratio. This demonstrates that fermentation substrate composition plays a crucial role in optimizing the functional quality of nata, especially in terms of its crude fiber enrichment.

Research Implications

The findings of this study contribute significantly to the optimization of nata production in the food industry, particularly through the utilization of organic waste materials such as watermelon rind, molasses, and mung bean sprout broth. The significantly high crude fiber content (as observed in N2 treatment) underscores the potential of nata derived from watermelon rind as a functional food product rich in dietary fiber. Dietary fiber is well known to support digestive health by increasing fecal bulk and regulating gastrointestinal activity [31]. In line with the growing demand for environmentally friendly production practices, these results offer a biotechnology-based solution that not only adds value to food waste but also aligns with the principles of green chemistry.

Furthermore, the study holds pedagogical relevance, particularly for the implementation of the Kurikulum

Merdeka, which promotes contextual learning through Project-Based Learning (PjBL). This approach fosters the development of 21st-century skills, including critical thinking, creativity, collaboration, and communication [32][33]. The findings can be translated into the development of Student Activity Sheets for school chemistry, incorporating local issues such as the fermentation of watermelon rind waste, grounded in microbiology and green chemistry concepts. Contextual and project-based learning approaches are considered effective for providing more meaningful learning experiences by connecting science content to students' everyday lives [34]. In addition, environmentally oriented PjBL has been shown to effectively enhance science process skills, such as observation, classification, prediction, data interpretation, and scientific communication [35].

Conclusion

Based on the research findings, variations in the volume of molasses and mung bean sprout broth in fermentation media made from watermelon rind significantly influenced the crude fiber content of the resulting nata. Treatments with 5% (N1) and 10% (N2) additions of molasses and mung bean sprout broth led to a significant increase in crude fiber content compared to the control (N0), which used coconut water as the fermentation medium. Treatment N2 yielded the highest crude fiber content, at 8.05 ± 0.02%, indicating that a more balanced combination of carbon and nitrogen nutrients enhanced the cellulose synthesis activity of *Acetobacter xylinum*. These findings underscore the potential of utilizing watermelon rind as a sustainable raw material for producing high-value functional nata while contributing to organic waste reduction through eco-friendly biotechnology. Furthermore, the research has educational relevance within high school chemistry instruction through the application of Project-Based Learning (PjBL). These hands-on activities enable learners to explore fermentation variables and relate their findings to green chemistry principles and sustainability issues. Such learning projects not only deepen students' scientific understanding but also foster 21st-century skills and environmental awareness aligned with the Kurikulum Merdeka.

Author's Contribution

Muhammad Ramadhan Cholili, Fifijihana Dewirukmana Putri, Khansa Farahdilla Irfanindya, and Natasya Kharisma Putri were responsible for conducting the research, collecting data, and drafting the manuscript. Guntur Trimulyono contributed to data validation and provided technical assistance during the laboratory analysis. Achmad Lutfi served as the primary supervisor, overseeing the entire research process and providing guidance and critical revisions during the manuscript preparation.

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