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Optimization of Functional Properties of Cocoyoghurt through Fortification with Moringa Leaf Extract (*Moringa oleifera*): Antioxidant Activity and Organoleptic Characteristics

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Abstract: Yogurt is a fermented milk product known as a functional food. Food diversification efforts by finding alternative yogurt raw materials to replace animal milk are necessary to provide products for people who want alternative non-dairy yogurt products. One of the plant-based milks that has a nutritional content similar to animal milk is coconut (Cocos nucifera). Moringa leaves (Moringa oleifera) are reported to contain antioxidant compounds and have therapeutic benefits, making them potentially suitable for use as a fortifier in cocoyogurt functional drinks. The purpose of this study was to obtain the most appropriate formula for adding moringa leaf extract to produce cocoyogurt products that are high in antioxidants and have physicochemical and organoleptic properties that are acceptable to consumers. The design used in this study was a completely randomized design (CRD) with 4 treatments, namely: P0 = without the addition of moringa leaf extract; P1 = moringa leaf extract 1 g/L; P2 = moringa leaf extract 2 g/L; and P3 = moringa leaf extract 3 g/L. The data obtained were analyzed using the Analysis of Variance (ANOVA) statistical method and continued with Duncan's Multiple Range Test if there was a significant difference ($P \leq 0.05$) between treatments. Based on the results of the study, it is known that the addition of moringa leaf extract to coconut milk yogurt produces cocoyoghurt with a low acidity (pH) (4.5-44.3), which is in accordance with the SNI standard (2981-2009) for yogurt quality. Overall, the best treatment in cocoyoghurt was the addition of 3 g/L of moringa leaf extract with an IC50 value of 148.33 µg/ml, with free radical inhibitory activity in the moderate category, and the sensory properties of taste, aroma, color, and texture showed the most preferred values by the panellists. These findings have broader implications for functional food innovation, particularly in developing plant-based, nutrient-enriched products that cater to lactose-intolerant populations and health-conscious consumers seeking natural sources of antioxidants.

Keywords: Coconut; Yoghurt; Moringa Leaf; Functional Food.

Introduction

Consumer demand for functional food products continues to rise in line with growing awareness of the importance of a healthy lifestyle. One rapidly developing product in this category is plant-based yoghurt, such as cocoyoghurt, which is yoghurt made from coconut milk. Cocoyoghurt has gained attention as an alternative for individuals with lactose intolerance and is known to contain healthy fats and dietary fiber beneficial for digestive health [1]. Coconut milk is particularly advantageous due to its fatty acid profile. It contains short- and medium-chain saturated fatty acids that have been clinically proven to help prevent hyperlipidemia, fatty liver, and diabetes, such as caproic acid (6.46%), caprylic acid (5.52%), capric acid (0.11%), lauric acid (50.45%), and myristic acid (17.52%) [2].

However, the antioxidant content in cocoyoghurt remains relatively low compared to fermented products fortified with bioactive compounds. On the other hand, research that specifically optimizes the addition of *Moringa oleifera* extract in coconut-based yogurt (*Cocos nucifera*), especially regarding its effect on antioxidant activity, is still very limited. This gap is significant, as both ingredients have high functional potential: coconut milk as a source of plant-based fat and moringa as a natural antioxidant, yet their optimal combination and formulation in fermented yogurt products remain underexplored. Several studies in the past five years have investigated the development of plant-based yogurts, such as one that formulated almond-based yogurt enriched with probiotics [3], and another that optimized soy yogurt fortified with green tea extract for antioxidant enhancement [4]. Furthermore, other research has shown that combining plant ingredients with bioactive compounds can improve the nutritional and stability profiles of fermented products [5]. However, to date, no comprehensive study has integrated moringa extract into coconut yogurt using an experimental design approach to simultaneously evaluate physicochemical, functional, and sensory parameters. Therefore, this study contributes new insights to the literature by systematically formulating and assessing moringa-fortified cocoyoghurt as an innovative, functional food product based on locally sourced ingredients.

Moringa leaves contain various bioactive compounds such as alkaloids, flavonoids, phenolics,

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triterpenoids/steroids, and tannins, which function as anticancer and antibacterial agents [6]. Additionally, moringa leaves contain higher levels of vitamin A, vitamin C, and calcium than oranges, carrots, and spinach. The phytochemical compounds in moringa leaves are classified as antioxidants, capable of scavenging free radicals and significantly protecting body cells from oxidative damage [7]. Research data show that fortifying yoghurt with moringa leaf extract significantly increases total phenolic content (TPC) and antioxidant capacity, without reducing product stability during storage, and even maintains antioxidant potential for up to eight weeks [8]. Furthermore, moringa leaf extract enhances antioxidant activity against free radicals such as DPPH and ABTS.

In addition to increasing antioxidant activity, the addition of moringa leaf extract to cocoyoghurt must also consider organoleptic aspects such as taste, flavor, color, and texture to ensure consumer acceptability. Therefore, a comprehensive study is needed to evaluate the effect of moringa leaf extract fortification on the functional and sensory qualities of cocoyoghurt.

This study aims to optimize the functional properties of cocoyoghurt through the addition of moringa leaf extract, with a focus on enhancing antioxidant activity and evaluating organoleptic characteristics. The findings from this research are expected to serve as a foundation for developing innovative, health-oriented plant-based functional food products.

Research Methods

The design used in this study was a single-factorial Completely Randomized Design (CRD) with five treatments:

1. P0 = 0% Moringa leaf extract
2. P1 = 5% Moringa leaf extract
3. P2 = 10% Moringa leaf extract
4. P3 = 15% Moringa leaf extract
5. P4 = 20% Moringa leaf extract

Each treatment was carried out four (4) times, resulting in 20 experimental units. The data obtained were analyzed using the Analysis of Variance (ANOVA) statistical method and continued with Duncan's Multiple Range Test if there was a significant difference ($P \leq 0.05$) between treatments.

Bacteria Stater Refreshment

Refreshment of y*ogourmet* brand bacterial cultures (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*) was carried out by inoculating the bacterial culture into sterilized UHT milk using a 1:9 ratio. The milk was previously sterilized using an autoclave at 115°C for 3 minutes. The milk inoculated with the LAB starter was then incubated at 37°C for 18 hours until coagulation formed. This rejuvenation process was conducted to cultivate and adapt the starter to the medium before the actual fermentation process.

Preparation of Coconut Milk

A total of 900 g of finely chopped coconut was mixed with 1800 mL of water at 60°C (1:2 ratio), blended until smooth, and poured into a container to be squeezed using a filter cloth. The resulting coconut milk was stored in a covered container until further use [9].

Preparation of Moringa Leaf Extract

The preparation of moringa leaf extract began by separating the moringa leaves from their stems and washing them thoroughly. The leaves were air-dried for 3 days, avoiding direct sunlight. They were then oven-dried at 130°C for approximately 10 minutes to remove residual moisture. The dried moringa leaves were ground into a fine powder [9].

Preparation of Cocoyoghurt Fortified with Moringa Leaf Extract

Coconut milk was poured into glass bottles, with each bottle containing 300 mL. Each bottle was supplemented with 15% skim milk and 5% sucrose. Moringa leaf extract was added according to the treatment levels: 1 g, 2 g, and 3 g. The mixture was homogenized and then pasteurized at 80°C for 10 minutes. After pasteurization, it was cooled to 45°C, followed by inoculation with 5% *Yogourmet* starter culture (*L. bulgaricus*, *S. thermophilus*, and *L. acidophilus*). The inoculated mixture was then incubated at 40°C for 8 hours [9].

Analytical Procedures

pH Measurement

The pH value was measured using a pH meter. The device was calibrated using standard buffer solutions at pH 4 and 7. The electrode was inserted into the yoghurt sample and allowed to stabilize until the reading on the pH meter no longer changed.

Antioxidant Activity Test (IC_{50} Method)

A 0.1 mM DPPH solution was prepared by dissolving 0.004 g of DPPH in a 100 mL volumetric flask, then adding methanol up to the mark. Sample concentrations were varied at 0, 100, 200, 300, 400, and 500 mg/mL. From each concentration, 0.5 mL was taken and mixed with 1 mL of 0.1 mM DPPH solution. Then, 4 mL of methanol was added, the mixture was vortexed until homogeneous, and incubated for 30 minutes. Absorbance was measured at a wavelength of 517 nm [10].

Organoleptic Test

The organoleptic evaluation assessed preferences for taste, aroma, color, and texture. A semi-trained panel of 30 male and female university students participated. Samples were coded and presented randomly. Panellists were asked to rate their level of preference for each sensory attribute. A 7-point hedonic scale was used: 1) Dislike very much; 2) Dislike; 3) Slightly dislike; 4) Neutral; 5) Slightly like; 6) Like; 7) Like very much [11].

Results and Discussion

pH Test

The pH test in yoghurt is crucial for assessing the quality and safety of the product, as it reflects the level of acidity generated during fermentation. In this study, the pH of cocoyoghurt fortified with moringa leaf extract ranged between 4.5 and 4.3 (Figure 1), aligning with the Indonesian National Standard (SNI 2981-2009), which stipulates that yoghurt should have a pH between 4.0 and 4.6. This condition indicates an optimal acidic profile for a functional yoghurt product [12].

The decrease in pH is primarily attributed to the production of lactic acid by lactic acid bacteria (LAB). *Streptococcus thermophilus* initiates the fermentation process by lowering the pH to around 5.5, followed by *Lactobacillus delbrueckii* subsp. *bulgaricus*, which further reduces the pH to around 4.3–4.6 [13]. Studies have shown that a final pH value of approximately 4.36 is commonly found in yoghurt fermented with a combination of *L. plantarum* and *S. thermophilus*, both in commercial products [14] and research settings [15].

During fermentation, the metabolic activity of LAB leads to the formation of organic acids derived from the breakdown of lactose and other simple carbohydrates [16]. These acids dissociate and release H^+ ions, which significantly contribute to the decline in pH. The longer the fermentation and the more active the bacteria, the greater the accumulation of H^+ ions, resulting in lower pH levels and enhanced microbiological stability as well as natural preservative properties in the yoghurt [17].

Interestingly, LAB are acid-tolerant and can grow optimally in low pH conditions (around 3.7-4.3), allowing them to remain active and beneficial even in more acidic environments. However, if the pH falls below their tolerance threshold, their growth may be inhibited. Therefore, optimizing the formulation and fermentation time is essential to maintaining a balanced acid–microbial interaction [18].

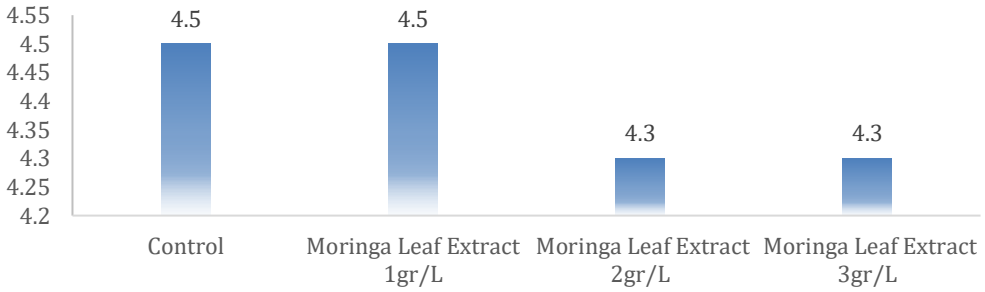

Figure 1. pH Test Results of Cocoyoghurt Fortified with Moringa Leaf Extract

Antioxidant Activity (IC_{50})

Statistical analysis using ANOVA showed that all formulations had a significant effect at $P < 0.05$ on the antioxidant activity of cocoyogurt fortified with moringa leaf extract. The results of the antioxidant activity analysis are listed in Table 1. Table 1 shows that the antioxidant activity of cocoyoghurt increases with higher concentrations of moringa leaf extract. This is supported by the presence of various bioactive compounds in moringa leaves, such as ascorbic acid, flavonoids, phenolics, and carotenoids, which play a significant role in neutralizing free radicals [19].

One study reported that moringa leaf extract contains a total phenolic content of up to 54.5 mg GAE/g, with IC_{50} values of 60 mg/L against ABTS radicals and 133 mg/L against DPPH radicals [8]. Although cocoyogurt fortified with 3 g/L of moringa leaf extract showed antioxidant activity with an IC_{50} value of 148.33 µg/mL, which is still in the moderate category, this value is significantly lower than pure moringa extract which was able to achieve an IC_{50} of 49.9–50.59 µg/mL, which is included in the strong antioxidant activity category [20][21]. This difference is likely due to the interaction between the yogurt matrix and the bioactive compounds from moringa, which can reduce the effectiveness of antioxidants by binding to the yogurt's proteins or fats. Furthermore, the mixing and fermentation processes can also reduce the bioavailability of active compounds, either through compound degradation or changes in their chemical structure. For comparison, another study on a water-based moringa drink showed an IC_{50} value against DPPH radicals of 75 µg/mL, indicating higher effectiveness than moringa cocoyogurt. This indicates that the protein- and fat-free liquid matrix allows antioxidant compounds such as polyphenols and flavonoids to remain in their free form and be more readily available to scavenge free radicals [22]

Although the antioxidant effectiveness of Moringa cocoyogurt is not yet comparable to its pure extract, the potential of *Moringa oleifera* leaves as a fortification ingredient in yogurt products remains very promising. Various studies have confirmed that Moringa is rich in bioactive compounds such as flavonoids, phenolics, tannins, vitamin C, and carotenoids, which play an important role in warding off free radicals and inhibiting oxidative stress. Research by Saini et al [23] states that Moringa leaves contain total phenolics up to 54.5 mg GAE/g, with an IC_{50} value against DPPH reaching 49.9 µg/mL, which is classified as a strong antioxidant activity. This content makes Moringa very promising as a natural functional agent in fermented foods such as yogurt.

Fortifying yogurt with moringa not only adds nutritional value but can also improve oxidative stability and enrich the product's functional profile, especially as consumers increasingly seek food alternatives that are not only delicious but also beneficial for health. Moringa has immunoprotective, anti-inflammatory, and antimicrobial effects, making it more than just a source of antioxidants [24]. In the context of yogurt, the addition of moringa extract

was also shown not to significantly impair the growth of lactic acid bacteria, which are key to probiotic viability during fermentation [25].

Table 1. IC_{50} Antioxidant Activity of Cocoyoghurt Fortified with Moringa Leaf Extract

Formulation	Antioxidant Activity (µg/ml)	Interpretation
Control	300.56 ± 1.58[d]	Sangat Lemah
1gr/L	197.00 ±1.00[c]	Lemah
2gr/L	184.97 ± 1.15[b]	Lemah
3gr/L	148.33 ±2.51[a]*	Sedang

Description:
1. Data are presented as mean ± standard deviation (SD) of 4 replicates
2. [ab]Data followed by the same letter in the same column are not significantly different in the Duncan test at the 0.05 level.
3. *sign indicates the highest antioxidant activity

However, the success of moringa fortification in yogurt is strongly influenced by the product matrix. Fat and protein components in yogurt can interact with bioactive compounds, causing a decrease in antioxidant effectiveness, as reflected in the IC_{50} value of moringa cocoyogurt (148.33 µg/mL). Therefore, a formulation optimization approach is important, for example, through the use of encapsulation techniques, pH modification, or adjustment of extraction methods (ultrasonic, microwave-assisted extraction) to maintain the stability and bioactivity of active compounds.

Furthermore, the success of this formulation also opens up opportunities for the development of innovative and competitive locally-based functional foods. Moringa is a plant that thrives in Indonesia and has long been recognized in traditional medicine. This potential can be further developed through further research evaluating the stability of active compounds during storage, the viability of probiotics in the final product, and sensory acceptance by consumers across various segments. With the support of scientific research and appropriate technological approaches, moringa can play a significant role as a local bioactive agent in the development of functional yogurt that is healthy and has high added value.

Table 2. Organoleptic Test Results of Cocoyoghurt Fortified with Moringa Leaf Extract

Parameter	Control	Moringa Leaf Extract		
		1gr/L	2gr/L	3gr/L
Rasa	5.10±0.26[a]	5.45±0.20[b]	5.40±0.37[ab]	5.33±0.40 [ab]
Aroma	5.85±0.35[a]	5.55±0.36[b]	5.52±0.23[b]	5.47±0.28[b]
Warna	4.50±0.25[a]	5.78±0.19[b]	5.92±0.10 [b]	6.36±0.49[c]
Tekstur	5.24±0.17[a]	5.28±0.70[a]	5.46±0.17[a]	5.40±0.33[a]

1. Data are presented as mean ± standard deviation (SD) of 4 replicates
2. [ab]Data followed by the same letter in the same column are not significantly different in the Duncan test at the 0.05 level.

Organoleptic Test

The ANOVA test results showed that the cocoyogurt formulation fortified with moringa leaf extract was significantly different ($P < 0.05$) in terms of color attributes, while the formulation showed no significant difference in aroma, texture, and taste attributes ($P > 0.05$). The results of the cocoyogurt fortified with moringa leaf extract are presented in Table 2.

The organoleptic test using a hedonic scale based on the five senses provides a direct indication of how much a product is liked by consumers. In this study, cocoyoghurt with added moringa leaf extract (1 g/L, 2 g/L, and 3 g/L) showed average scores ranging from 5.33 to 6.36 on a 7-point scale, indicating a preference level from "dislike very much" to "like very much." The highest scores were given to the 3 g/L treatment, particularly for color (6.36), while taste, aroma, and texture remained within an acceptable range (5.4–5.5) (Table 2).

These findings are consistent with a study on soymilk yoghurt fortified with moringa root powder, where certain formulations remained acceptable to panellists, although the control sample without extract was generally preferred [26]. Another study noted that while moringa fortification enriches nutritional value, sensory effects such as a green herbal color may reduce preference at higher concentrations [27].

In their recent review, Azis et al [28] examined various studies from 2014 to 2024 on the fortification of yogurt with *Moringa oleifera* leaf extract or powder. They noted that concentrations up to 0.5% significantly enhanced total phenolic content, antioxidant activity (DPPH, ABTS), water holding capacity (WHC), and yogurt viscosity, while maintaining acceptable pH levels and probiotic viability. However, at higher concentrations, undesirable sensory characteristics such as an astringent taste and greenish coloration began to appear, which could reduce consumer acceptability. This supports that the 3 g/L (~0.3%) concentration used in this study is sufficiently optimal in enhancing color and antioxidant activity without compromising palatability.

The decrease in organoleptic scores (particularly for taste, aroma, and texture) despite an increase in color with the addition of 3 g/L of moringa leaf extract can be explained by the distinctive sensory characteristics of moringa leaves, which begin to dominate the product at high concentrations. Moringa leaf extract is known to contain phytochemical compounds such as flavonoids, tannins, and saponins, which, in addition to acting as antioxidants, also impart a bitter, tart, or astringent flavor that can reduce consumer acceptance. Some of these compounds also have a strong herbal aroma, which is tolerable at low levels, but at higher concentrations becomes more easily detected and tends to be unpopular with panellists.

In terms of texture, the addition of extracts in liquid or powder form can also affect the viscosity and mouthfeel of the product, making it feel rougher or less smooth. This combination of less familiar flavor perceptions, sharper aromas, and potential changes in texture is why sensory scores other than color do not increase proportionally with increasing extract concentration.

However, although the addition of 3 g/L of moringa extract provided the highest contribution to antioxidant activity and attractive color appearance, this needs to be balanced with reformulation, for example, by adding natural flavors, sweeteners, or an encapsulation process of active compounds to suppress negative sensory impacts and maintain its functional quality.

Conclusion

Fortification of moringa leaf extract in coconut milk-based yogurt resulted in cocoyogurt with low acidity (pH 4.5–4.3), which meets the Indonesian National Standard (SNI 2981-2009) for yogurt quality requirements. Overall, the best treatment was observed in cocoyogurt fortified with 3 g/L moringa leaf extract, which achieved an IC_{50} value of 148.33 µg/mL—classified as having moderate free radical scavenging activity. In terms of sensory attributes, this formulation also received the highest preference scores from panellists for taste, aroma, color, and texture. This coconut milk-based product can be a suitable alternative for lactose-intolerant consumers who cannot consume yogurt made from animal milk. The cocoyogurt formulation with moringa leaf extract can be an effective case study for students and entrepreneurs in developing functional foods based on local commodities. This product combines the probiotic benefits of yogurt and antioxidants from moringa, and is relevant to healthy consumption trends. Future research can focus on storage stability, probiotic viability, and optimization of extraction methods to enhance the product's bioactive properties.

Author's Contribution

Putu Rima Sintyadewi: conceived and designed the study and finalized the manuscript. Ida Ayu Putu Ary Widnyani: performed the laboratory experiments and collected the data. A.A. Ngurah Dwi Ariesta Wijaya Putra: conducted the data analysis. Nadya Treesna Wulansari: assisted in sample preparation.

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