



Tropical Almond–Based Yogurt Fortified with Celery: A Functional Food for Lipid Profile Improvement and Anti-Inflammatory Support

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Abstract. *The development of yogurt as a functional food has gained significant attention. Tropical almond (*Terminalia catappa*) is rich in fat and protein, while celery is known for its antioxidant, anti-inflammatory, hypolipidemic, and hypoglycemic properties. This study investigated the development of functional yogurt made from tropical almond milk fortified with celery extract. It employed a Completely Randomized Design (CRD) with varying celery extract concentrations (1%, 2%, and 3%) and fermented for 14, 17, and 20 hours. The results showed that after 17 hours of fermentation, total acid-producing bacteria reached 7.87 log CFU (colony forming unit)/ml, exhibiting acceptable sensory attributes and enhanced antioxidant activity. In vivo tests on Wistar rats revealed significant reductions in malondialdehyde (MDA) and interleukin-6 (IL-6) levels, which indicate anti-inflammatory and lipid-lowering effects. These findings highlight the potential of tropical almond-based yogurt as a novel functional food, paving the way for sustainable dietary interventions targeting metabolic health.*

Keywords: *Tropical almond; celery; plant-based yogurt; malondialdehyde; inflammation.*

Type of the Paper: Regular Article.



1. Introduction

Yogurt is a food product produced through the fermentation of milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. While cow's milk is commonly used as a base, this study explores yogurt made from tropical almonds (*Terminalia catappa*). *Terminalia catappa* is a hardwood tree often used for shade; its seeds are rich in protein, ash, water, crude fiber, and vitamins C and B1–B3 [1]. The seeds also contain bioactive compounds such as quinine, ribalinidine, sapogenin, flavan-3-ol, catechin, and tannin. Their fat content includes beneficial fatty acids such as palmitic, oleic, linoleic, stearic, and myristic acids [2]. Tropical almonds have strong potential as a raw material for producing plant-based yogurt because of their high protein and saturated fatty acid content. Moreover, previous studies have reported adequate cell counts to classify the product as probiotic.

Previous studies have developed plant-based yogurts using various ingredients. Nasef et al. [3] used coconut extract, while Jamalullail et al. [4] used legumes. Suhartatik et al. [5] reported

that tropical almond-based yogurt has potential as a probiotic food, with cell counts ranging from 7.35 to 7.75 log CFU/ml. Continued innovation aims to enhance the functional properties of such products. Yogurt has also been enriched with various additives, including pineapple peel powder, grape extract, melon puree or powder, mango juice, lemon, pawpaw, orange juice, date juice, pumpkin, carrot, green peas, zucchini puree, chicory inulin extract, potato peel powder, green coffee powder, and green tea powder [6]. In this study, celery was used as an additive in tropical almond-based yogurt to further enhance its functional properties. The rationale for combining these two ingredients is based on their complementary potential to be developed into functional foods. Plant-based yogurt offers the advantage of being cholesterol-free and provides unsaturated fatty acids, while celery is recognized for its anti-inflammatory and antihypertensive properties [1]. When combined, these components are expected to yield a functional food product with dual benefits and, more importantly, the capacity to promote overall health. In support of this, Ramanan et al. [2] reported that the incorporation of additional bioactive ingredients into yogurt formulations can enhance health effects beyond those provided by yogurt alone, thereby reinforcing the value of combining tropical almond and celery in a single fermented matrix.

Celery (*Apium graveolens*) is a nutrient-rich vegetable with various health benefits. It contains bioactive compounds that exhibit antihypertensive effects, primarily through vasodilatory, diuretic, and calcium channel-blocking mechanisms [7]. Enzyme-treated celery extract has demonstrated anti-obesity effects in mice by reducing body weight gain, improving lipid profiles, and preventing insulin resistance [8]. Celery also shows antioxidant properties and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [9,10]. Its health-promoting effects are attributed to compounds such as apiin, apigenin, luteolin, and 3-n-butylphthalide [7,8,10]. According to Yusni et al. [8], celery leaves also contain natural phytosterols, which are known to reduce blood glucose levels. That is why celery leaves were selected as an additional ingredient in the formulation of this plant-based yogurt.

Tropical almond has potential as a functional food due to its rich nutritional content. Fortifying tropical almond-based yogurt with celery is expected to enhance its nutritional and functional value. The kernel of *Terminalia catappa* is a highly nutritious component of the fruit, offering substantial potential as a food ingredient. It contains approximately 25.42% protein and 52.02% lipids [2], making it a rich source of energy and essential nutrients. Although the protein content of tropical almonds is still lower than that of soybeans, which contain 39.4% protein [3], tropical almonds offer distinct advantages because they provide higher levels of omega-6 and omega-9 fatty acids compared to soybeans. The kernel is traditionally consumed fresh, roasted, or smoked, and its flour is used as a thickening agent in soups, baby food, and high-protein formulations. In Côte d'Ivoire, kernel flour has been incorporated into wheat-based cakes,

enhancing both the nutritional profile and sensory appeal of the product. Additionally, the kernel yields up to 65% edible oil, which meets dietary standards and is used for cooking in parts of South America.

Recent studies have highlighted the potential of yogurt consumption in improving lipid profiles. A double-blind controlled study showed that yogurt enriched with *Lactococcus lactis* 11/19-B1 and *Bifidobacterium lactis* significantly reduced LDL levels in individuals with high LDL [11]. Data from the Korean National Health and Nutrition Examination Survey indicated that consumption of viscous yogurt was associated with lower serum triglyceride levels among Korean adults [12]. Similarly, yogurt containing fermented pepper juice was found to reduce body fat accumulation and cholesterol levels in rats on a high-fat diet [13]. These findings suggest that yogurt, particularly when fortified with specific ingredients or probiotic strains, may support lipid metabolism and overall health, although further research is needed to confirm these effects in humans. Additionally, daily consumption of dairy products did not increase the risk of cardiovascular or liver disorders in hyperlipidemic rats and was associated with improved enzyme activity in the liver and heart [14].

Yogurt may also offer anti-inflammatory benefits. In a community-based study, yogurt consumption was linked to lower levels of inflammatory biomarkers such as interleukin-6 and fibrin [15]. A randomized controlled trial reported that daily low-fat yogurt consumption for nine weeks reduced chronic inflammation, including IL-6, hs-CRP, and TNF- α , in both lean and obese women. Biological activity testing of yogurt enriched with natural ingredients has been widely reported. For instance, yogurt supplemented with curcumin has demonstrated anti-inflammatory properties [4]. Yogurt fortified with black pepper juice has shown effects on body fat reduction and cholesterol modulation [5]. Further the incorporation of *Codonopsis pilosula*, *Illicium verum*, *Lycium barbarum*, and *Psidium guajava* has enhanced yogurt's antidiabetic and antioxidant activities [6] while *Portulaca oleracea* (purslane) has been investigated for its antioxidant, anticancer, antibacterial, and antiviral effects [7]. These findings underscore the versatility of yogurt as a carrier matrix for bioactive compounds, enabling the development of functional food products with diverse health-promoting properties. However, to date, no studies have examined the antioxidant, hypolipidemic, or anti-inflammatory potential of tropical almond-based yogurt fortified with celery, underscoring the novelty and significance of the present investigation.

While the benefits of yogurt as a functional food are well established, the functional potential of tropical almond-based yogurt remains underexplored. Whether this plant-based yogurt delivers effects comparable to conventional cow's milk yogurt warrants further investigation. Therefore, this study aims to examine the effects of consuming tropical almond-based yogurt with added celery on the blood profile of test animals, its antioxidant properties, and its potential as an anti-

inflammatory functional food. This study was designed as an exploratory investigation to evaluate the potential of celery extract in enhancing the functional properties of fermented tropical almond-based yogurt. The selection of extract concentrations and fermentation durations was based on preliminary laboratory trials, aimed at identifying feasible and effective parameters for product development. As such, the findings provide foundational insights for future optimization and scale-up studies. The findings are expected to contribute to the development of functional food products that may improve human health and well-being. This study introduces a novel formulation of plant-based yogurt using tropical almond milk fortified with celery extract, two-underutilized ingredients with complementary functional properties. To our knowledge, this is the first investigation to combine these components in a fermented matrix, demonstrating enhanced probiotic viability and bioactivity through *in vivo* validation. The product offers a sustainable, non-dairy alternative with potential anti-inflammatory and lipid-lowering effects, contributing to the advancement of functional food innovation.

2. Materials and Methods

2.1. *Preparation of tropical almond-based yogurt with celery extract*

Tropical almonds were obtained from local producers in Bekasi Province, Indonesia [5]. The seeds were de-husked after being soaked overnight in distilled water. The preparation of tropical almond-based milk followed the method by Suhartatik et al. [16], with some modifications. The seeds were split in half, cleaned, and blanched for 10 minutes. Fresh celery, purchased from a local market, was separated from its stems. The leaves were washed under running water, drained, and weighed. They were then blanched in boiling water for 5 minutes. The blanched tropical almond and celery were homogenized with distilled water at a 1:8 ratio. The resulting puree was filtered to obtain tropical almond-celery milk.

The concentrations of celery extract (1%, 2%, and 3%, v/v) and fermentation durations (14, 17, and 20 hours) used in this study were determined based on preliminary laboratory trials. These internal trials, although not formally published, provided empirical evidence for optimal yogurt formation and stability. Concentrations above 3% were found to inhibit yogurt formation, likely due to interference with microbial activity or protein coagulation. Similarly, the selected fermentation times were chosen to capture the critical window for microbial growth and product consistency, as observed during trial runs. These parameters were thus adopted to ensure the feasibility and reproducibility of the experimental design.

One hundred milliliters of tropical almond-celery milk was placed in a graduated cylinder and mixed with 10% (w/v) skim milk and 10% (w/v) sucrose. The mixture was homogenized and heated to approximately 85°C for 15 minutes. After cooling, 10% (w/v) of a commercial yogurt

starter culture was added. The mixture was incubated at 37°C for 14, 17, or 20 hours.

2.2. *Yogurt characterization*

Total acid-producing bacteria were quantified using the pour plate method on deMann Rogosa Sharpe (MRS) medium supplemented with 1% CaCO₃. Standard methods from the Association of Official Analytical Chemists (AOAC, 2005) were followed: total sugar was measured using the Nelson–Somogyi method [17], protein by the micro-Kjeldahl method, fat by the Mojonnier method, and ash by the thermogravimetric method. Antioxidant activity was assessed using the DPPH method [16,18], and flavonoid content was analyzed following the method described by El-Hawary et al. [19].

2.3. *Analytical methods*

2.3.1. *Total sugar*

Total sugar was analyzed using Nelson–Somogyi method [17]. The analysis of total sugar content began with the preparation of a standard curve showing the relationship between sugar concentration and absorbance. A total of 100 ml of sugar solution with a concentration of 10 mg anhydrous glucose per 100 ml was diluted to obtain concentrations ranging from 0.02 to 0.08 mg/ml. Then, 1 ml of Nelson's reagent was added to 1 ml of the sugar solution and heated in a water bath for 20 minutes. The solution was allowed to stand, after which 1 ml of arsenomolybdate reagent was added. The mixture was shaken until homogeneous, followed by the addition of 7 ml of distilled water, and shaken again. Calibration was performed at 540 nm using a spectrophotometer.

For sample treatment, 25 ml of filtrate was placed into an Erlenmeyer flask, followed by the addition of 15 ml of distilled water and 5 ml of concentrated HCl. The mixture was heated in a water bath at 67–70°C for 30 minutes, then rapidly cooled to 20°C. The solution was adjusted to neutral pH by adding 45% NaOH and diluted to a final volume of 100 ml, resulting in a solution containing 0–10 mg/ml of reducing sugar. The absorbance values were then subjected to linear regression analysis to determine the relationship between concentration and absorbance.

2.3.2. *Total protein*

Total nitrogen or crude protein was analyzed using micro-Kjeldahl method. Protein content was determined using the Micro-Kjeldahl method. Approximately 0.5–1.0 g of homogenized sample was weighed into a Kjeldahl digestion flask, followed by the addition of 10 ml concentrated sulfuric acid (H₂SO₄) and a catalyst mixture (potassium sulfate and copper sulfate or selenium) to accelerate digestion. The flask was heated gently at first and then at higher temperature until the solution became clear, indicating complete digestion of organic matter. After cooling, 50 ml of distilled water was added, and the digest was transferred into a distillation apparatus. The solution

was neutralized with excess sodium hydroxide (NaOH, ~40%) to release ammonia, which was distilled into a receiving flask containing 2% boric acid solution with mixed indicator. Distillation was continued until all ammonia was collected. The boric acid solution containing absorbed ammonia was then titrated with standardized hydrochloric acid (HCl) or sulfuric acid (H₂SO₄) until the endpoint was reached. The nitrogen content was calculated based on the titration results using the formula (1).

$$\%N = \frac{(V \times N \times 14.007)}{W} \times 100 \quad (1)$$

Where V is the titrant volume, N is the normality of the acid, and W is the sample weight. Protein content was obtained by multiplying the nitrogen percentage with the conversion factor 6.25.

2.3.3. *Fats*

Total fat was determined using the Mojonnier flask method. Approximately 3–5 g of homogenized sample was weighed into a Mojonnier fat-extraction flask. To facilitate protein dissolution and phase separation, 10 ml of ammonium hydroxide was added and the mixture was gently swirled. The sample was then extracted with 25 ml of ethanol, followed by the addition of 25 ml of diethyl ether and 25 ml of petroleum ether. The flask was stoppered and shaken vigorously for 1–2 minutes, then allowed to stand until clear phase separation occurred. The upper organic (ether) layer containing dissolved lipids was carefully decanted into a pre-weighed fat beaker. A second and third extraction were performed by adding fresh portions of diethyl ether and petroleum ether (typically 15–20 ml each), with vigorous shaking and decanting to the same beaker to ensure exhaustive lipid recovery. The combined extracts were rinsed from the walls of the beaker with small volumes of solvent and then evaporated on a steam bath to remove any residual ether. The beaker was placed in an oven at 100–105°C until a constant mass was achieved, cooled in a desiccator, and weighed. Fat content was calculated based on the mass of residue relative to the original sample mass (2).

$$\% Fats = \frac{(Mb - Me)}{M_{sample}} \times 100 \quad (2)$$

Where Mb was beaker + fats weigh; Me = weigh of empty beaker, and M sample = weigh of the sample

For quality assurance, glassware was conditioned to constant weight, and duplicate determinations were conducted to verify repeatability.

2.3.4. *Ash content*

Total ash was determined using the thermogravimetric method. Approximately 2–5 g of homogenized sample was weighed into a pre-ignited, pre-weighed porcelain crucible. The sample was dried at 105°C to remove moisture, cooled in a desiccator, and reweighed to ensure accurate

dry mass. The crucible was then placed in a muffle furnace and incinerated at 500–600°C until a light gray or white residue was obtained, indicating complete oxidation of organic matter (typically 4–6 hours, or until constant weight). After incineration, the crucible was cooled in a desiccator and weighed. Ash content was calculated using equation (3).

$$\% \text{ Ash} = \frac{(M' - Mo)}{Ms} \times 100 \quad (3)$$

Where M' is weigh of crucible and ash; Mo = weigh of crucible; and Ms = weigh of crucible + sample. Quality controls included running reagent blanks if applicable, conditioning crucibles to constant weight, and performing duplicate determinations to confirm repeatability.

2.3.5. Antioxidant activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [16,18]. A stock solution of DPPH was prepared by dissolving 0.1 mM DPPH in methanol and stored in the dark until use. For the assay, 1 ml of sample extract was mixed with 3 ml of the DPPH solution and incubated at room temperature in the dark for 30 minutes to allow the reaction to occur. The decrease in absorbance was measured at 517 nm using a UV–Vis spectrophotometer, with methanol serving as the blank. The radical scavenging activity was calculated as a percentage of inhibition using the formula (4).

$$\% \text{ RSA DPPH} = \frac{Ac - As}{Ac} \times 100 \quad (4)$$

Where *Ac* is the absorbance of the DPPH solution without sample and *As* is the absorbance in the presence of the sample. Greater inhibition values indicate stronger antioxidant activity. All measurements were performed in triplicate to ensure reproducibility.

2.3.6. Flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method described by El-Hawary et al. [19]. Briefly, 1 ml of sample extract was mixed with 4 ml of distilled water in a test tube, followed by the addition of 0.3 ml of 5% sodium nitrite solution. After 5 minutes, 0.3 ml of 10% aluminum chloride solution was added, and the mixture was allowed to stand for another 6 minutes. Subsequently, 2 ml of 1 M sodium hydroxide was added, and the final volume was adjusted to 10 ml with distilled water. The solution was thoroughly mixed, and absorbance was measured at 510 nm using a UV–Vis spectrophotometer. Quercetin was used as the standard reference compound, and a calibration curve was prepared with concentrations ranging from 0 to 100 µg/ml. Flavonoid content was expressed as milligrams of quercetin equivalent (mg QE) per milliliter of extract. All measurements were performed in triplicate to ensure accuracy and reproducibility.

2.4. *Animal experimental*

Thirty male Sprague Dawley rats (*Rattus norvegicus*), aged 7 weeks, were obtained from the Clinical Evaluation Unit for Animal Research at Gadjah Mada University, where the experimental procedures were also conducted. All procedures involving animal subjects were conducted in accordance with ethical standards and were approved by the ethics committee of Dr. Moewardi Hospital, Surakarta, Indonesia, under approval number 2.012/IX/HREC/2025. The study protocol was reviewed and granted ethical clearance prior to implementation, ensuring compliance with institutional and international guideline for the care and use of laboratory animals. A total of six rats were randomly assigned to each treatment group (n = 6 per group). The bioassay procedures followed the methods described by Zhang et al. and Santoso et al. [20,21]. The random allocation of animals was carried out using a simple randomization method to reduce selection bias and ensure balanced distribution across treatments.

Group 1 (G1) consisted of healthy rats fed standard feed without any treatment. Group 2 (G2) included hyperlipidemic rats without treatment, while Group 3 (G3) comprised hyperlipidemic rats treated with the standard drug simvastatin. Groups 4 to 6 (G4–G6) consisted of hyperlipidemic rats treated with tropical almond-based yogurt (0.2 ml per day). Hyperlipidemia was induced over one week through a high-fat diet (lard), and lipid profile tests were conducted at the end of the induction period. Hyperlipidemia in rats was defined as total cholesterol at least twice the minimum standard 200 mg/dL. Product administration continued for four weeks (28 days), with blood sampling performed twice per week. Blood plasma was analyzed for lipid profiles, interleukin-6 (IL-6), and malondialdehyde (MDA) levels. Lipid profiles including total cholesterol, total HDL, and total LDL using CHOD-PAP enzymatic photometric test while total glyceride using GPO-PAP enzymatic photometric test [22]. Serum concentration of Interleukin 6 (IL-6) was measured by human IL-6 Quantikine ELISA kit from bio-technie (RnD Systems, Mineapolis, MN). Methods were described by the manufacturer. MDA level based on the reaction with thiobarbituric acid (TBA) [23].

2.5. *Statistical analysis*

The experimental data were analyzed using a two-factorial design under a Completely Randomized Design (CRD) to assess statistical significance. The triplicate findings were analyzed using ANOVA to investigate the variability between data means at the confidence level of 95%. SPSS 27.0 software (IBM< Armonk, New York, USA) was used for the statistical analysis.

3. Results and Discussion

3.1. *Acid-producing bacteria*

Lactic acid bacteria are the primary microorganisms responsible for yogurt fermentation.

These bacteria thrive under microaerophilic conditions and are characterized by their catalase-negative nature. The total number of acid-producing bacteria in celery tropical almond-based yogurt is presented in Table 1. The bacterial count ranged from 6.39 to 7.87 log colony-forming units (CFU)/ml. The highest bacterial count (7.87 log CFU/ml) was observed in yogurt containing 2% celery extract and fermented for 17 hours. In contrast, the lowest count (6.39 log CFU/ml) was found in yogurt with 3% celery extract fermented for 20 hours. This variation indicates that both the concentration of celery and the fermentation duration influence the growth of acid-producing bacteria in the yogurt.

Table 1. Characteristics of celery-Tropical almond yogurt

% celery & fermentation time	Cell count (log CFU/ml)	Protein (%)	Fat (%)	Sugar (%)	DPPH radical scavenging activity (%)	Flavonoid mg QE/ml
14h						
1% of celery	6.92	4.20±0.04 ^b	3.50±0.21 ^a	8.25±0.04 ^f	29.06±0.00 ^b	0.10±0.00 ^{bc}
2% of celery	7.60	4.42±0.10 ^c	3.80±0.14 ^{ab}	8.97±0.02 ^g	29.77±0.00 ^d	0.23±0.00 ^e
3% of celery	6.80	4.50±0.09 ^c	4.63±0.10 ^c	9.14±0.03 ^g	30.52±0.00 ^f	0.32±0.00 ^f
17h						
1% of celery	6.54	4.79±0.05 ^d	3.98±0.01 ^b	5.33±0.02 ^d	28.67±0.00 ^a	0.06±0.00 ^{ab}
2% of celery	7.87	4.13±0.01 ^b	3.97±0.14 ^b	5.87±0.01 ^e	29.58±0.00 ^{cd}	0.17±0.00 ^d
3% of celery	6.91	4.80±0.08 ^d	4.73±0.20 ^c	4.83±0.05 ^c	30.32±0.00 ^{ef}	0.25±0.00 ^e
20h						
1% of celery	7.25	4.10±0.02 ^b	3.80±0.13 ^{ab}	2.80±0.08 ^a	28.60±0.01 ^a	0.05±0.00 ^a
2% of celery	7.16	3.86±0.08 ^a	4.10±0.06 ^b	2.97±0.14 ^{ab}	29.38±0.00 ^c	0.17±0.00 ^d
3% of celery	6.39	4.57±0.13 ^c	3.78±0.19 ^{ab}	3.15±0.00 ^b	30.13±0.00 ^e	0.15±0.00 ^{cd}

Note: Values followed by different superscripted letters within the same column are significantly different ($p \leq 0.05$)

In general, acid-producing bacteria increased with longer fermentation times. However, in this study, the bacterial count in celery tropical almond-based yogurt did not differ significantly across treatments. The pH value of the celery tropical almond-based yogurt ranged from 4.1 to 4.4. Szoltysik et al. [24] reported that the addition of honeysuckle berries extracts for some treatments did not significantly affect total *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Yogurt with 3% celery extract exhibited a lower bacterial count than yogurt with 2% celery. This may be attributed to the antimicrobial properties of celery, which is known to act against a broad range of pathogens, including Gram-positive and Gram-negative bacteria as well as fungi [25,26]. Bioactive compounds in celery, such as flavonoids, saponins, and tannins, may inhibit the growth of lactic acid bacteria in yogurt. At a concentration of 2%, celery extract may not exert strong antimicrobial effects, allowing for higher bacterial growth. Celery (*Apium graveolens* L.) contains bioactive compounds such as flavonoids, polyphenols, alkaloids, and terpenoids that exhibit antimicrobial activity through multiple mechanisms. These compounds disrupt microbial cell membranes via hydrophobic interactions, leading to leakage of cellular contents and loss of membrane integrity. Flavonoids like apigenin and luteolin also chelate metal ions and inhibit key

microbial enzymes, impairing cell wall synthesis and replication. Additionally, phenolics induce oxidative stress by generating reactive oxygen species (ROS), overwhelming microbial antioxidant defenses and contributing to cell death [27].

3.2. Protein

Protein is a key macronutrient essential for energy production, tissue building, and regulatory functions in the human body. Protein in yogurt contributes to its textural and functional properties. The protein content in celery-tropical almond-based yogurt ranged from 3.86% to 4.80% as shown in Table 1, depending on the concentration of celery extract and fermentation duration. The highest protein level (4.80%) was recorded in yogurt containing 3% celery extract fermented for 17 hours, while the lowest (3.86%) was found in yogurt with 2% celery extract fermented for 20 hours. Statistical analysis using ANOVA confirmed that both celery concentration and fermentation time had a statistically significant effect on total protein content ($p \leq 0.05$), indicating that these variables play a decisive role in protein retention and transformation during fermentation.

Celery extract contains bioactive compounds, such as flavonoids and saponins, which may interact with proteins during fermentation, potentially enhancing protein stability or altering solubility. Fermentation time influences microbial activity, particularly proteolytic enzymes produced by lactic acid bacteria (LAB). These enzymes can hydrolyze proteins into peptides and amino acids, which may be detected as increased protein content depending on the analytical method used (e.g., micro-Kjeldahl). The protein levels observed in this study are comparable to those reported in other plant-based yogurts, such as legume-based yogurt, which typically 2.5% [4]. According to Indonesian National Standard (SNI 2981.2009), the minimum protein content for yogurt is 2.7%. All samples in this study exceeded this threshold, affirming their nutritional adequacy.

3.3. Fat

Fat in yogurt plays a dual role: it contributes to mouthfeel and energy density, while also influencing its health implications. In this study, the lipid content of tropical almond-based yogurt ranged from 3.50% to 4.73% as shown in Table 1, depending on the concentration of celery extract and fermentation duration. ANOVA results indicated that both celery concentration and fermentation duration significantly affected lipid content, suggesting that a dynamic interaction between formulation and microbial activity influenced lipid content ($p \leq 0.05$). The highest fat content (4.73%) was observed in yogurt with 3% celery extract fermented for 17 hours, while the lowest (3.50%) was found in yogurt with 1% celery extract fermented for 14 hours. According to the Indonesian National Standard (SNI) 2981:2009, the minimum fat content required for yogurt is 3.0%. Thus, all yogurt samples in this study met the national standard. Yogurt fat levels can be

influenced by medium composition, initial fat content, and bacterial development during fermentation. This aligns with the findings of Wajs et al. [6], who reported that higher initial fat content in the medium leads to increased fat content in the final yogurt product. Tropical almond contains 19.84% fat per 100 g [28], which likely contributed to the fat content observed in the yogurt samples. Compared to conventional dairy yogurt (typically 3-4% fat), the lipid content in this plant-based formulation remains within a nutrition acceptable range, especially for consumer seeking low-fat yogurt. The tropical almond base contributes unsaturated fatty acids, which are known for their cardioprotective effects, distinguishing this yogurt from saturated-fat-rich dairy products.

3.4. *Sugars*

The sugar content in yogurt formulations plays a pivotal role not only in sensory attributes but also in microbial dynamics and functional properties. In this study, the total sugar content of celery-tropical almond-based yogurt ranged from 2.80% to 9.14%, with the highest concentration observed in samples containing 3% celery extract fermented for 14 hours, and the lowest in those with 1% extract fermented for 20 hours. These findings corroborate previous research by Marlapati et al. [29], which demonstrated that the sugar content in plant-based yogurt ranges from 9.3% to 12.8% for soybean, oat, almond, cashew, and coconut yogurts, respectively.

This inverse relationship between fermentation duration and sugar concentration aligns with established principles of lactic acid bacteria (LAB) metabolism. LAB utilize available sugars, primarily sucrose added during formulation, as a carbon source, converting them into lactic acid and other metabolites. As fermentation progresses, the depletion of sugars is expected due to microbial consumption, which also contributes to acidification and pH reduction.

Interestingly, the highest sugar content was retained in the shortest fermentation time (14 hours), suggesting limited microbial activity and incomplete sugar utilization. Conversely, extended fermentation (20 hours) resulted in significantly lower sugar levels, indicating more advanced microbial metabolism. This trend is critical for product optimization, especially in functional food development where sugar content must be balanced against probiotic viability and health claims.

Moreover, the presence of celery extract may influence sugar metabolism indirectly. Celery contains bioactive compounds such as flavonoids and saponins, which exhibit antimicrobial properties [30]. At higher concentrations, these compounds may partially inhibit LAB activity, thereby modulating sugar consumption rates. However, the data suggest that at 3% extract concentration, the inhibitory effect is not sufficient to prevent sugar degradation during longer fermentation, as evidenced by the reduced sugar content at 20 hours.

3.5. *Antioxidant activity*

Previous studies have explored the enrichment of yogurt with various plant extracts to enhance its functional and sensory qualities. The addition of herbal extracts such as thistle, hawthorn, sage, and marjoram has been shown to improve yogurt's physicochemical and rheological properties while increasing its antioxidant activity [31]. Similarly, incorporating rosemary, dill, oregano, and ginger extracts significantly enhance α -amylase inhibition and antioxidant capacity [18]. Supplementing yogurt with aronia juice has also been reported to improve antioxidant potential and flavor, with the best taste observed at a 2% concentration [32]. These findings demonstrate the potential of plant extract-enriched yogurts to serve as functional food products with health-promoting and improved sensory attributes.

In this study, the antioxidant activity of celery-tropical almond-based yogurt ranged from 28.60% to 30.52%. The highest antioxidant activity (30.52%) was observed in yogurt containing 3% celery extract and fermented for 14 hours, while the lowest (28.60%) was found in the 1% celery treatment fermented for 20 hours. Increased celery concentration led to higher antioxidant activity, indicating that celery contributes significantly to the yogurt's functional properties. Celery contains a variety of antioxidant compounds, including flavonoids, saponins, tannins, riboflavin, flavo-glucosides, apigenin, phytosterols, choline, lipase, asparagine, and vitamins A, B1, C, and K, as well as nicotinamide [33]. These compounds likely contributed to the enhanced antioxidant potential observed in celery-tropical almond-based yogurt.

3.6. *Total flavonoid*

Flavonoids are widely distributed in nature, with more than 9,000 types identified to date [34]. These compounds play important roles in neutralizing free radicals, inhibiting hydrolytic and oxidative enzymes, and acting as anti-inflammatory agents. Structurally, flavonoids are polyphenolic compounds consisting of 15 carbon atoms arranged in a C6-C3-C6 configuration, two substituted benzene rings (C6) connected by a three-carbon aliphatic chain (C3). In this study, the total flavonoid content in yogurt increased with higher concentrations of celery extract. Additionally, several studies have noted that fermentation can enhance flavonoid levels. This is due to enzymes produced by lactic acid bacteria (LAB) that degrade polyphenols. During fermentation, LAB produce sugar-degrading enzymes that not only break down complex sugars like sucrose into glucose and fructose but also degrade phenolic compounds and release phenolic moieties from the substrate, leading to the formation of additional flavonoid compounds [35].

The increase in flavonoid content observed with higher celery extract concentrations is particularly relevant to the functional properties of the yogurt. Flavonoids are known to exert strong antioxidant and anti-inflammatory effects by scavenging free radicals, inhibiting lipid peroxidation, and modulating inflammatory pathways such as NF- κ B. In this study, the elevated

flavonoid levels are consistent with the reductions in malondialdehyde (MDA) and interleukin-6 (IL-6) observed *in vivo*, suggesting that flavonoids contributed to both the antioxidant and anti-inflammatory effects. Fermentation may enhance flavonoid bioavailability through enzymatic hydrolysis by lactic acid bacteria, which release phenolic moieties from bound forms. Thus, the tropical almond–celery yogurt not only provides a plant-based source of protein and unsaturated fatty acids but also delivers bioactive flavonoids that support lipid profile improvement and inflammation control. These findings highlight the relevance of flavonoid enrichment in positioning the product as a functional food with measurable health benefits.

3.7. Total cholesterol

The total cholesterol levels of hyperlipidemic rats ranged from 193.122 to 201.362 mg/dL, whereas healthy rats had significantly lower levels, at 83.762 mg/dL as shown in Fig. 1. Administration of celery-tropical almond-based yogurt resulted in a reduction in total cholesterol among the treated groups. Higher concentrations of celery extract corresponded to greater reductions in cholesterol. Notably, yogurt containing 3% celery extract produced cholesterol-lowering effects comparable to those of the standard drug, simvastatin.

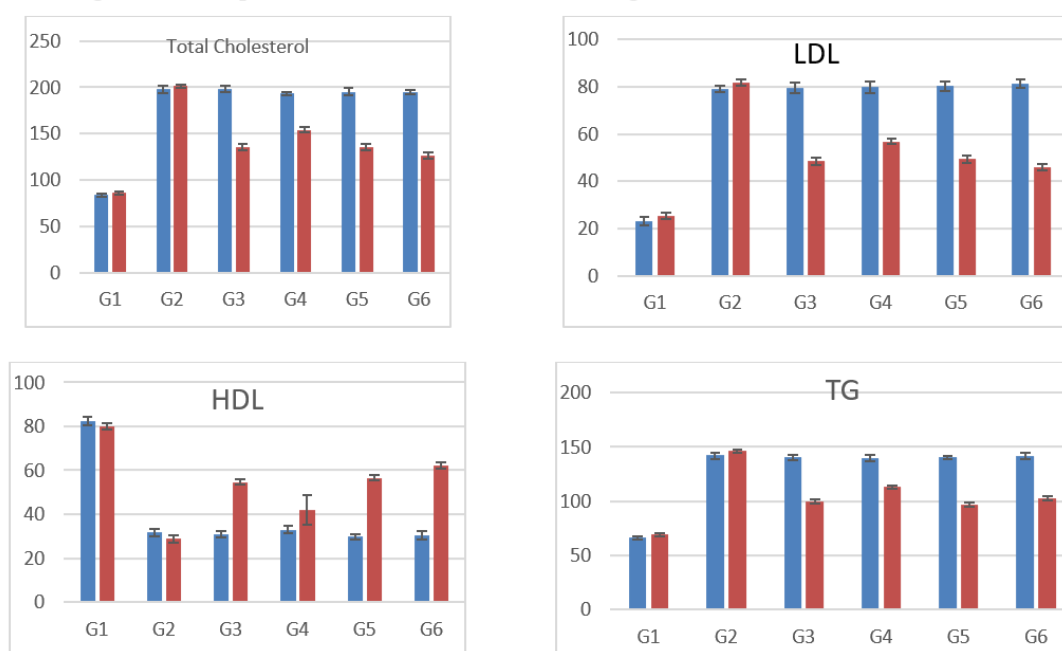


Fig. 1. Lipid profile of blood plasma, before and after treatment (total cholesterol, LDL, HDL, and TG present in mg/dL blood plasma) (blue: before treatment; red: after treatment). G1 for healthy groups; G2 negative control; G3 positive control; G3-G6 yogurt with 1%, 2%, and 3% celery

A similar trend was observed in low-density lipoprotein (LDL) levels as shown in Fig. 1. Rats treated with yogurt showed lower LDL levels compared to untreated hyperlipidemic rats, with those receiving 3% celery yogurt displaying LDL levels nearly equivalent to those in the simvastatin-treated group. High-density lipoprotein (HDL) levels also improved in the yogurt-treated groups, although they did not reach the levels observed in healthy rats as shown in Fig. 1.

However, rats given celery-tropical almond-based yogurt showed higher HDL levels than those treated with simvastatin, indicating a potentially more favorable effect on lipid profiles.

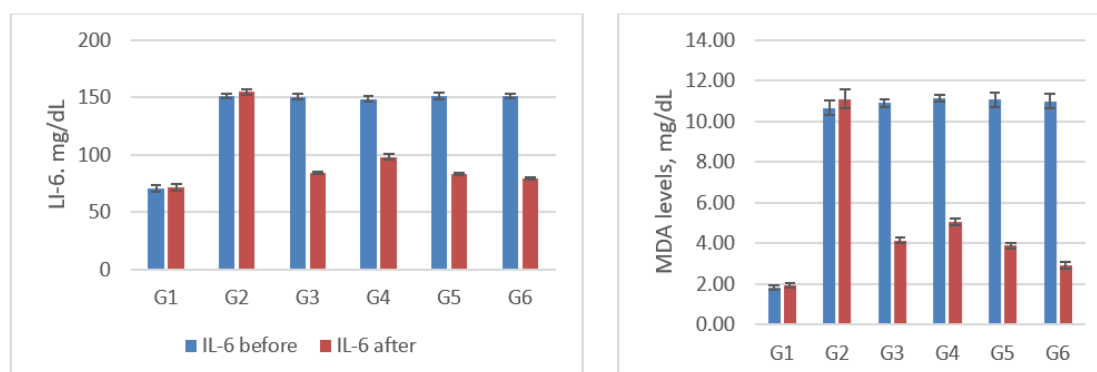
Regarding triglycerides, initial levels after hyperlipidemia induction were 66.27 mg/dL in the healthy group, 142.1420 mg/dL in group G2 (untreated hyperlipidemic rats), and 140.22 mg/dL in group G3 (simvastatin-treated). Groups G4, G5, and G6—treated with celery-tropical almond-based yogurt—had triglyceride levels of 139.78 mg/dL, 140.52 mg/dL, and 141.40 mg/dL, respectively. After 28 days of treatment, triglyceride levels slightly decreased to 69.23 mg/dL in the healthy group, 146.3625 mg/dL in group G2, and 99.70 mg/dL in group G3. Although reductions were also seen in the yogurt-treated groups, the differences were not statistically significant, suggesting that celery-tropical almond-based yogurt may have a limited effect on lowering plasma triglyceride levels.

This limitation in the impact could be explained by several factors. First, triglyceride metabolism is influenced by hepatic synthesis and peripheral lipoprotein lipase activity, processes that are less directly modulated by flavonoids and phytosterols compared to cholesterol metabolism. While celery contains bioactive compounds with hypolipidemic potential, their primary mechanism involves inhibition of HMG-CoA reductase and reduction of cholesterol absorption rather than suppression of triglyceride synthesis. Second, the almond base contributes unsaturated fatty acids (oleic and linoleic acids) that are cardioprotective but can be metabolized into triglycerides under certain dietary conditions, thereby attenuating the net TG-lowering effect. Third, the duration of intervention (28 days) may not have been sufficient to observe significant changes in triglyceride turnover, which often requires longer dietary modulation. Finally, animal models of diet-induced hyperlipidemia typically exhibit more pronounced elevations in cholesterol than triglycerides, which may explain the stronger response in cholesterol parameters. These factors suggest that while celery–tropical almond-based yogurt is effective in improving cholesterol profiles, its influence on triglyceride metabolism is more limited and may require formulation adjustments or longer intervention periods to achieve measurable reductions.

3.8. *IL-6*

Interleukin-6 (IL-6) levels (Fig. 2) were measured in rat groups following hyperlipidemia induction. In the normal group, IL-6 levels were 70.80 mg/dL, while levels in G1 and G2 were 151.13 mg/dL and 150.54 mg/dL, respectively. In the treatment groups, IL-6 levels were 148.48 mg/dL for G4 (1% celery), 151.13 mg/dL for G5 (2% celery), and 151.62 mg/dL for G6 (3% celery). After 28 days of celery-tropical almond-based yogurt administration, IL-6 levels in the normal group (G1) were 71.78 mg/dL. The hyperlipidemic control group (G2) showed an increase to 155.06 mg/dL, while the simvastatin-treated group recorded 84.56 mg/dL. The yogurt-treated groups showed progressively lower IL-6 levels with increasing celery concentrations: 98.33 mg/dL

(1%), 83.28 mg/dL (2%), and 79.55 mg/dL (3%). Although the IL-6 level in the 3% celery group remained slightly higher than that of the healthy group, it was lower than the simvastatin group and not significantly different from normal levels. These results suggest that increasing celery concentration in yogurt correlates with greater anti-inflammatory effects, as indicated by the reduction in IL-6 levels.



(blue: before treatment; red: after treatment; G1 for healthy groups; G2 negative control; G3 positive control; G3-G6 yogurt with 1%, 2%, and 3% celery)

Fig. 2. IL-6 levels (mg/dL) in blood plasma

Yogurt consumption, particularly dairy-based, has been associated with reduced levels of inflammatory markers such as IL-6 and fibrin in adults [15]. Celery extract has demonstrated potent anti-inflammatory activity, being five times more effective than celery stem extract in inhibiting LPS-induced nitric oxide production in RAW 264.7 cells [36]. These anti-inflammatory effects have been linked to modulation of arginine metabolism, specifically involving the TCA and urea cycles. Bioactive compounds such as apigenin and bergapten have been identified as key contributors to celery's anti-inflammatory properties [36].

3.9. MDA

Malondialdehyde (MDA) is a well-established biomarker for lipid peroxidation, reflecting the extent of oxidative degradation of unsaturated fatty acids. Elevated MDA levels are associated with cellular damage and inflammation, making it a critical parameter in evaluating the health-promoting potential of functional foods. Following hyperlipidemia conditioning, the malondialdehyde (MDA) level in the healthy rat group was 1.84 mg/dL. In contrast, the hyperlipidemic group (G2) showed an MDA level of 10.66 mg/dL, while the group receiving standard drug treatment (G3) recorded 10.90 mg/dL (Fig. 2). After 28 days, MDA levels in the healthy rats (G1) remained stable, whereas levels in the untreated hyperlipidemic group increased slightly to 11.10 mg/dL. Rats treated with simvastatin and those receiving celery-tropical almond-based yogurt showed a reduction in MDA levels. The most significant decrease was observed in the group treated with yogurt containing 3% celery extract, followed by the groups receiving 2% and 1% celery yogurt, respectively. These results indicate that celery-tropical almond-based yogurt may contribute to reducing oxidative stress. Overall, the findings support the potential of yogurt

enriched with celery to provide antioxidant and anti-inflammatory benefits. However, further studies are necessary to elucidate the specific mechanisms by which this product influences LDL cholesterol and overall lipid profiles.

The reduction in MDA levels with increasing celery concentration can be attributed to the presence of flavonoids, polyphenols, and vitamin C in celery, which are known to scavenge free radicals and inhibit lipid peroxidation. These compounds likely interact with reactive oxygen species (ROS), preventing the formation of MDA as a secondary oxidation product [37]. Fermentation time also plays a pivotal role [38]. Shorter fermentation (14 hours) appears to preserve antioxidant compounds more effectively, while extended fermentation (20 hours) may lead to partial degradation of these bioactive or increased oxidative stress due to prolonged microbial metabolism. This aligns with findings from previous studies on plant-based fermented products, where antioxidant capacity tends to peak at intermediate fermentation durations.

The observed reduction in IL-6 and MDA levels in rats fed with tropical almond–celery yogurt may be attributed to the synergistic action of flavonoids and phenolic compounds present in celery extract. Flavonoids such as apigenin and luteolin are known to inhibit the NF- κ B signaling pathway, a key regulator of pro-inflammatory cytokine production including IL-6. By suppressing this pathway, celery-derived compounds likely contributed to the downregulation of systemic inflammation. Additionally, the antioxidant properties of celery—mediated through scavenging of reactive oxygen species (ROS)—may explain the decreased MDA levels, a marker of lipid peroxidation. These mechanisms suggest that the functional yogurt not only supports probiotic viability but also exerts measurable anti-inflammatory and antioxidative effects, reinforcing its potential as a therapeutic dietary intervention.

4. Conclusions

This study demonstrates the successful development of a plant-based functional yogurt using tropical almond milk fortified with celery leaf extract. The formulation exhibited favorable physicochemical properties, including adequate protein and lipid content, while maintaining acceptable sugar levels across varying fermentation durations. Notably, the yogurt showed promising bioactive potential, with significant antioxidant activity, reduced malondialdehyde (MDA) levels, and improved inflammatory and lipid profiles in hyper-lipidemic rat effects that were comparable to simvastatin treatment.

These findings highlight the synergistic role of tropical almond and celery in enhancing both nutritional and therapeutic attributes of fermented products. By integrating locally sourced botanical ingredients with probiotic fermentation, this research contributes to the growing field of plant-based functional foods aimed at supporting cardiometabolic health. Future studies should

explore sensory evaluation, shelf-life stability, and clinical validation to further establish the product's applicability in broader dietary interventions. Overall, this yogurt formulation offers a compelling alternative to conventional dairy-based products, aligning with global trends in sustainable nutrition and preventive healthcare.

Abbreviations

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CFU	Colony Forming Unit
CHOP-PAP	Cholesterol Oxidase – Peroxidase Amino-antipyrine
CRD	Completely Randomized Design
DPPH	2,2-difenil-1-pikrilhidrazil
ELISA	Enzyme-linked Immunosorbent Assay
GPO-PAP	Glycerol Peroxidase Phosphoric Acid
HDL	High Density Lipoprotein
Hs-CRP	High-sensitivity C-Reactive Protein
IL-6	Interleukin 6
LAB	Lactic Acid Bacteria
LDL	Low Density Lipoprotein
MDA	Malondialdehyde
MRS	DeMann Rogosa Sharpe
QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
TBA	Thio-barbituric Acid
TNF- α ,	Tumor Necrosis Factor-alpha
SNI	Standar Nasional Indonesia (Indonesian National Standard)

Data Availability Statement

Data will be made available on request.

CRedit Authorship Contribution Statement

Soraya Habibi: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing-Original Draft, Writing-Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition. **Einstivina Nuryandani:** Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing-Original Draft, Writing-Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition. **Nanik Suhartatik:** Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing-Original Draft, Writing-Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition. **Merkuria Karyantina:** Conceptualization, Methodology, Validation, Formal Analysis, Writing-Review & Editing, Supervision. **Fadilah Husnun:** Conceptualization, Methodology, Validation, Formal Analysis, Writing-Review & Editing, Supervision. **Akhmad Mustofa:** Conceptualization, Methodology, Validation, Formal Analysis, Writing-Review & Editing, Supervision. **Pundhi Ludiani Hartiwi:** Methodology, Formal Analysis, Investigation,

Writing-Original Draft, Writing-Review & Editing, Visualization.

Declaration of Competing Interest

The authors declare no competing interests.

Declaration of Use of AI in the Writing Process

Nothing to disclose.

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