

Peanut Milk Vegan Cheese Using Pineapple Bromelain as a Coagulant and Carrot Extract for Antioxidant Enrichment

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Abstract: Cheese, a fermented food, has been consumed since ancient Greece. Cheese is obtained through a protein coagulation process facilitated by bacteria, enzymes, and acids. Through the advancement of technology, various types of processed cheese have been created. Cheese is often produced using milk from cows, goats, horses, and several other animals. Nevertheless, the occurrence of advancements in the production of cheese from herbs or plants is quite uncommon. The development of a new plant-based cheese product involves using peanut milk combined with pineapple bromelain as a coagulant. Additionally, the use of carrots serves to enhance the cheese's antioxidant properties. This study aims to assess the sensory attributes of peanut-based vegan cheese products through organoleptic testing, measure their protein content using the biuret method, and evaluate their antioxidant properties using DPPH solution. Three samples subjected to these tests comprised sample 1, a control sample of a peanut milk vegan cheese product without carrot extract; sample 2, which contained 15% carrot extract; and sample 3, which contained 30% carrot extract. Of the three tests conducted, sample 1 outperformed in terms of texture in organoleptic tests of cheese and had a protein level of 17%. On the other hand, sample 2 exceeded in terms of the average acceptance of organoleptic tests, particularly in the scent and taste of cheese. Furthermore, sample 3 outperformed the others in terms of color in organoleptic tests and had a significant level of antioxidant activity in cheese. Based on the quality assessment of the peanut milk vegan cheese, it can be concluded that this product had the potential to serve as a substitute for vegetable-based cheese product.

Keywords: carrot extract; peanut milk; pineapple bromelain; quality test; vegan cheese

1. Introduction

Cheese is generally produced from fermented cow's milk through the process of protein coagulation facilitated

by microbes, enzymes, and acids. Nevertheless, milk, a frequently used ingredient in cheese production, can cause allergic reactions in certain individuals. Dairy products and their derivatives are the main cause of allergic reactions, representing 35% of all reported cases (Prawibowo, 2021). Cow's milk cheese is rich in fat and cholesterol, which might negatively affect one's health. The solution to addressing the issue of allergenic cheese and promoting a more body-friendly option is to substitute the protein found in cow's milk with vegan milk alternatives, such as peanut milk. Peanuts contain approximately 27% protein with a complete amino acid composition, making them comparable to animal protein (Apriani et al., 2021). The cheesemaking process involves numerous distinct steps, including acidification, coagulation, dehydration, followed by shaping and pressing of the curd (Kartawiria et al., 2019). Rennet, obtained from the abomasum of young ruminants, serves as a source of protease enzymes. The renin enzyme exhibits optimal activity under acidic conditions, characterized by low pH values (Atang & Wardhono, 2019). The use of rennet derived from animal sources can significantly impact the adherence to halal standards in cheese products. In addition, extracting rennet from young animals potentially disrupts the growth of cattle as milk and meat producers, which possess greater economic value (Komansilan et al., 2023).

Rennet is extensively used in the food industry as a coagulant for milk in the production of cheese. However, its supply is limited, necessitating the use of substitute substances with similar coagulation properties. Moreover, the use of rennet in the cheesemaking process compels vegetarians to avoid consuming the product. Pineapple, scientifically known as *Ananas comosus*, is a substance that possesses protease enzymes that can act as a coagulant. Pineapple includes bromelain, a protease enzyme capable of coagulating milk protein into cheese. In addition, pineapple contains citric acid, which potentially stimulates the bromelain enzyme (Raisanti et al., 2022). Bromelain was selected as a component in pineapple due to its high abundance, making it readily available at a relatively low price. Bromelain functions through catalyzing the hydrolysis of proteins into amino acids. Pineapple's bromelain enzyme is commonly used for meat tenderization, but it can also be used to coagulate milk protein, a crucial process in cheese production (Komansila et al., 2020).

Cheese is rich in various nutrients, such as protein, fat, calcium, phosphorus, iron, riboflavin, thiamine, and several other vitamins. Riboflavin and thiamine are B vitamins with antioxidant effects, despite their frequently deficient concentrations. The thiamine in cheese is 0.06 grams per 100 grams (Chairunnisa et al., 2021). Antioxidants, on the other hand, protect the body against free radicals, which cause a variety of ailments. Scientific evidence has demonstrated that natural antioxidants provide protection against cancer and cardiovascular disease. Vegetables and fruits are rich sources of the most significant natural antioxidants (Parcheta et al., 2021). Regrettably, the consumption of fruits and vegetables as a means of obtaining antioxidants is exceedingly low in Indonesia. Based on the statistics from Riskesdas (2007), the Indonesian population aged 10 years exhibited a tendency to have lower levels of consumption. This is exemplified by the 93.6% of individuals who consume fewer than 5 servings of fruits and vegetables per week. In 2013, the percentage decreased slightly to 93.5%; however, by 2018, it had increased to 95.5%. Such increase was accompanied by a decrease in the consumption of vegetables and fruits (Woisiri et al., 2022). Carrots are one of the most common vegetables in Indonesia. It provides essential nutrients promoting good health, including vitamin A, fiber, minerals, and beta-carotene, which acts as an antioxidant (Lidiyawati et al., 2013). The state of the art of this research lies on the addition of carrot extract, which serves as a source of antioxidants, into the production of peanut-made vegan cheese coagulated using pineapple bromelain. This study aims to assess the sensory attributes of peanut-based vegan cheese products through organoleptic testing, measure their protein content using the biuret method, and evaluate their antioxidant properties using DPPH solution on three samples.

2. Methodology

2.1. Equipment and Materials

The investigation used many instruments, including a beaker, dropper pipette, test tube, measuring flask, vortex mixer, stopwatch, spectrophotometer, blender, basin, stove, filter cloth, and measuring cup. The materials used in this research comprised of 1 kilogram of peanuts, water, 500 grams of pineapple, 250 grams of carrots, salt, biuret reagent, standard protein solution, NaOH, and distilled water.

2.2. Procedure

First, 500 grams of peanuts were gathered and soaked them in 1000 milliliters of water for an hour to facilitate the process of separating the outer layer. The shelled beans were boiled in water at a temperature of 90-100 °C until they were fully cooked. Subsequently, the water was drained. The nuts were mashed using a blender by adding an adequate amount of water. Next, the substance was heated at a temperature of 80–85 °C until reaching the boiling point and then left them at room temperature as it became warm. The coagulant enzyme was made from 500 grams of pineapple by mashing them to extract the juice. Afterwards, the enzyme was mixed with boiled peanut milk that had been left to reach a warm temperature so as to let the mixture fermented for 24 hours until it separated into two distinct layers. The succeeding phases were to divide the upper and lower layers and extract the

upper layer to drain it to separate the curds and whey. The curd was placed in a packaging container. Subsequently, a series of organoleptic, protein content, and antioxidant tests were conducted.

So as to achieve a high-quality final product, it is necessary to implement the quality control for both the raw materials and the use of instruments. Ensuring the quality of raw materials in cheese production involved verifying that the primary and secondary ingredients used were fresh, mature, and free from decay. In addition, it is also necessary to ensure the use of standard units, specifically grams for solid materials and liters for liquid materials. Quality control in the use of instruments encompassed their sterility. It was also beneficial for reducing work-related accidents during the production of vegan cheese. Figure 1 shows the schematic flowchart of the research procedure.

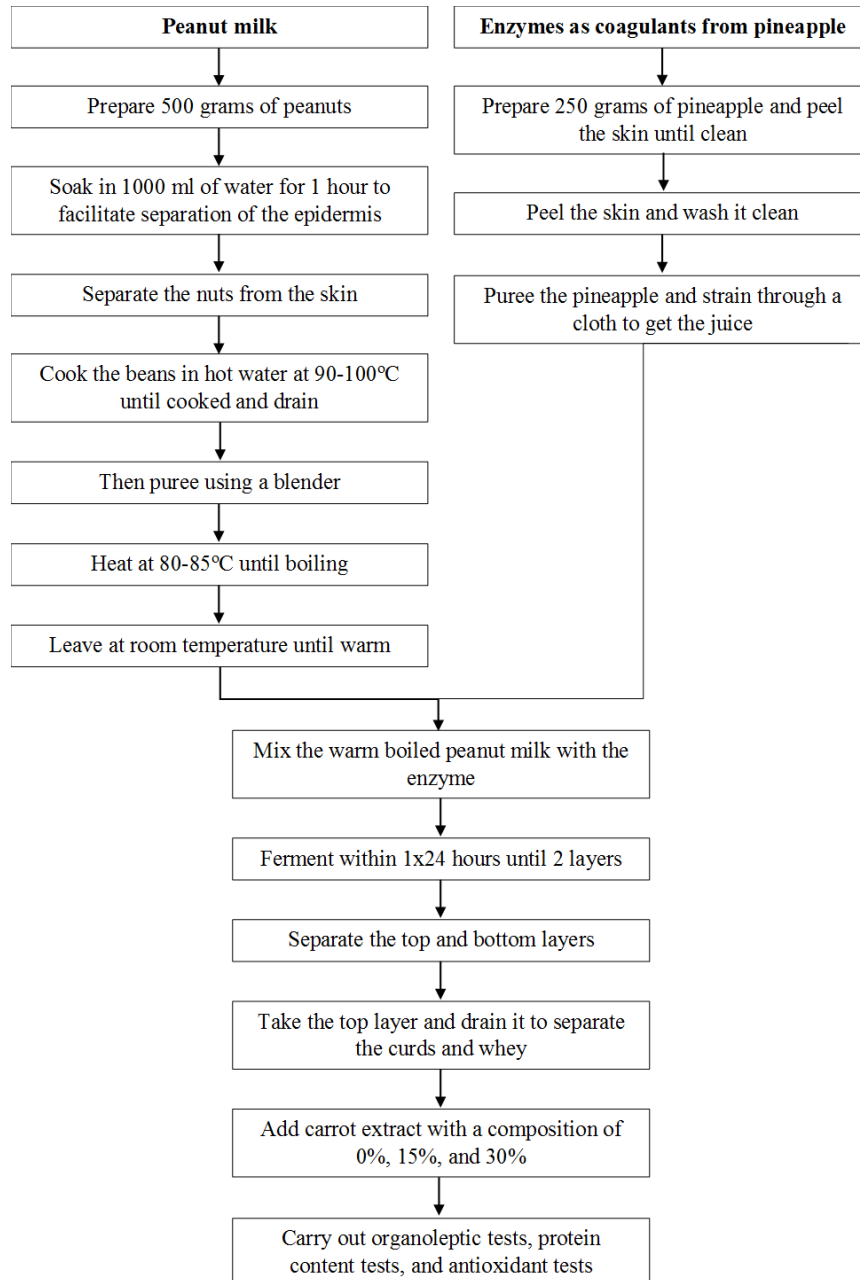


Figure 1. Schematic Flowchart of the Research Procedure

2.3. Organoleptic Test

The organoleptic test in this study was carried out on a group of biology education students from Universitas Negeri Surabaya using the scoring method to evaluate the intensity of several attributes of vegan peanut cheese, including taste, scent, color, and texture. The cheese was prepared using the coagulant enzyme bromelain, as well as extracts from pineapple and carrot. Organoleptic analysis was conducted on three distinct samples, varying in the concentration of carrot extract in peanut essence vegan cheese. Sample 1 contained no carrot extract, sample

2 contained 15% carrot extract, and sample 3 contained 30% carrot extract. Table 1 shows the rating scale of the organoleptic test.

Table 1. The Rating Scale Of The Organoleptic Test.

Levels of Pleasure	Numeric Scale
Very dislike	1
Do not like	2
Quite like	3
Like	4
Really like	5

2.4. Protein Level Test

The procedures for the protein level test are as follows:

1. Preparation of a protein standard solution for a standard curve with concentrations of 0.2; 0.4; 0.6; 0.8; 1 mg/ml egg white
10 ml of distilled water was prepared in 5 test tubes. Egg whites weighing 0.2, 0.4, 0.6, 0.8, and 1 ml were put in a test tube by means of a pipette. Afterwards, the egg white solution was homogenized using a vortex mixer for 30 seconds.
2. Sample preparation 0;15; and 30 ml
1 ml of each sample was collected and diluted with 10 ml of distilled water. The sample solution was homogenized using a vortex mixer for 30 seconds. This process was repeated for a total of 5 dilutions.
3. Sample measurement and Standard Solution
1 ml of prepared sample was put into a test tube, followed by adding the blank with distilled water therein. Subsequently, 4 ml of biuret reagent was added and homogenized using a vortex. It was allowed to stand for 30 seconds, and the absorbance was measured at the maximum wavelength.

2.5. Antioxidant Test

The procedures for the antioxidant test are as follows:

1. Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution
The DPPH powder was accurately measured to a maximum of 0.007 grams, which was then dissolved in 50 ml of ethanol and vigorously mixed until completely dissolved. In addition, 1 ml of DPPH solution was taken and then added with ethanol until the total volume reached 5 ml. The mixture was then allowed to stand for 30 minutes.
2. Determination of the maximum absorption wavelength
The solution was extracted using a pipette, collecting up to 1 ml, which was subsequently combined with 5 ml of ethanol solution. The resulting mixture was then incubated for 30 minutes in a dark environment. Next, the absorption was measured at the maximum wavelength.
3. Sample measurement
0.2 ml of each sample was taken, and 3.8 ml of DPPH was added. Afterwards, the mixture was homogenized and incubated for 30 minutes. The absorbance was measured at a wavelength of 516 nm.

3. Results and Discussion

Peanut vegan cheese is a dairy-free cheese produced using peanut milk. Vegan cheese was produced by using bromelain derived from pineapple as a coagulant. It was enriched with carrot extract, which serves as an antioxidant.

3.1 Organoleptic Test

An organoleptic test is a testing method that relies on the human senses as the primary means of measuring the acceptability of a product. The sensory attributes were evaluated using the senses of sight, touch, smell, and taste. An organoleptic test is crucial as it can be used to measure the level of consumer acceptance and preference for a product. The scoring test for intensity was employed to evaluate vegan cheese made with pineapple bromelain coagulants and carrot extract. The test encompassed flavor, scent, color, and texture. It was conducted on several panelists using three distinct samples based on the levels of carrot extract in peanut essence vegan cheese. Sample 1 contained no carrot extract, sample 2 contained 15% carrot extract, and sample 3 contained 30% carrot extract. Table 2 shows the organoleptic test results.

Table 2. The Organoleptic Test Results.

Sample	Parameters				Mean ± SD
	Taste	Scent	Color	Texture	
Sample 1	3,73	3,43	4,13	4,03	3,83 ± 0,32
Sample 2	3,86	3,56	4,16	3,9	3,87 ± 0,25
Sample 3	3,63	3,33	4,33	3,7	3,75 ± 0,42

3.1.1. Taste Organoleptic Test

The taste of a product was evaluated using the gustatory sense, specifically the tongue. When food encountered the taste buds, they can discern various types of food. The resulting taste can be influenced by the combination of ingredients used (Sylvia et al., 2019). The organoleptic test results indicated that, on average, the panelists expressed a preference for sample 2, which contained 15% carrot extract. The taste of the peanut cheese was regarded as distinct and savory due to the presence of peanuts. It had a not-to-strong salty taste. The panelists judged that this cheese was a suitable alternative to cow's milk cheese, possessing an equally delightful flavor.

3.1.2. Scent Organoleptic Test

Scent refers to the aroma generated from chemical stimulation in a product, which is detected by the olfactory nerves in the nasal cavity (Putri et al., 2022). The table show that the average panelist preferred the scent of sample 2 that contained 15% carrot extract. The scent of cheese was deemed moderately mild, as was the fragrance of nuts. There was a fresh aroma emanating from carrots.

3.1.3. Color Organoleptic Test

Color is a sensory instrument that influences the panelist's assessment of the product's acceptability. Product color testing relied on visual perception to determine the attractiveness of the product based on its color. The panelists, on average, preferred sample 3 that contained 30% carrot extract. The cheese was renowned for its vibrant color, specifically a light orange, which captivated consumers and piqued their curiosity to select it. This was because sample 3 had a higher quantity of carrots. Carrot extract can produce a visually appealing orange color due to the abundant carotene concentration found in carrots (Yoo et al., 2020).

3.1.4. Texture Organoleptic Test

Texture refers to the overall composition and consistency of a product, which can be evaluated through the touch sense. The texture of this product is characterized by the dense composition of the cheese. The water content can also influence the texture of the cheese. Water is a crucial component in food ingredients since it has the potential to influence the hardness, appearance, taste, and nutritional content of the meal (Indah et al., 2020). The table shows the average panelist preferred sample 1, which contained no carrot extract. The cheese's texture was comparable to spread cheese, although it was denser and more suitable for consumption than the other two samples. This was because the cheese was more fluid due to the presence of carrot extract.

Based on the organoleptic tests conducted, sample 2 was more preferred than the other two samples. The data shown in Table 2 indicated that sample 2 had an average acceptance level of 3.87 in the organoleptic test, with a standard deviation of 0.25. In contrast, sample 1 had an average of 3.83 with a standard deviation of 0.32 while sample 3 had an average of 3.75 with a standard deviation of 0.42.

3.2 Protein Content Test

Vegan peanut cheese, coagulated with bromelain pineapple and fortified with carrot extract for antioxidants, had a higher protein content than cow's milk cheese coagulated with the same coagulant. Cow's milk cheese with pineapple bromelain as a coagulant provides merely 12.64% protein per gram sample (Komansilan, 2020). Sample 1, cheese with 0% carrot extract, had the highest protein concentration at 17% per gram of sample. In contrast, sample 3, cheese with 30% carrot extract, had a lower protein content at 16% per gram of sample. Sample 2, cheese with 15% carrot extract, showed the lowest protein content at 15% per gram of the sample. The high protein content of the peanut raw material, which had a protein value of 24.41% per gram of sample (Trianto, 2019), cannot be separated from the overall high protein content.

After adding carrot extract, the protein level of peanut milk vegan cheese tended to decrease. The data clearly indicated that the addition of carrot extract to the two samples resulted in reduced protein levels compared to sample 1, which served as a control without the addition of carrot extract. However, the decrease in protein content was only 1-2%. Miwada et al. (2019) verified this finding, stating that adding purple sweet potato extract to goat

milk cheese as an antioxidant source led to a decrease in the cheese's protein level. The protein levels contained in vegan peanut cheese with pineapple bromelain coagulants and carrot extracts are shown in Table 3.

Table 3. Vegan Cheese Protein Level Test Results

Sample	Protein Content (g)	Percentage (%)
Sample 1	0.17 g	17%
Sample 2	0.15 g	15%
Sample 3	0.16 g	16%

3.3 Antioxidant Test

The vegan peanut cheese with pineapple bromelain coagulant and carrot extract had a significant number of antioxidants and produced satisfactory results. The addition of 0% carrot extract resulted in an IC₅₀ value of 137, 15% carrot extract and 30% carrot extract resulted in the IC₅₀ value of 115 and 70, respectively. The IC₅₀ value of each sample indicated this. The very high value of antioxidant activity content is indicated by IC < 50, 50 < IC₅₀ < 100 for high value, 100 < IC₅₀ < 150 for moderate value, and 150 < IC₅₀ < 200 for low value (Nasution et al., 2015). The antioxidant levels in vegan peanut cheese with pineapple bromelain as a coagulant and carrot extracts are indicated in Table 4.

Table 4. Vegan Cheese Antioxidant Test Results

Carrot Extract (%)	IC ₅₀ Score
0	137
15	115
30	70

The addition of carrot extract, which is rich in antioxidants, to peanut milk cheese was a deliberate attempt to enhance the nutritional content of the cheese, making it a functional food product. Carrots include many compounds that act as antioxidants, such as carotenoids, polyphenols, and vitamins (Varshney & Mishra, 2022). Antioxidants are compounds that help protect the body from harm caused by exposure to free radicals. Moreover, a consistent consumption of antioxidant compounds has the capacity to reduce the risk of acquiring fatal and degenerative illnesses (Jideani et al., 2021). Peanuts are a potential plant for a nutrient-rich food source that contains high levels of antioxidant from bioactive compounds, including phytosterols, phenolics, stilbenes, lignans, and isoflavonoids, which are bioactive in nature (Ciftci & Suna, 2022). This also supports the antioxidant content of peanut milk vegan cheese with the addition of carrot extract to reach the medium-to-high category.

Three tests were conducted on three vegan cheese samples, viz. sample 1 containing 0% carrot extract, sample 2 containing 15% carrot extract, and sample 3 containing 30% carrot extract. Among the three samples, sample 3 showed a higher antioxidant content compared to the other two samples. However, sample 1 had a higher protein content than sample 3. Moreover, sample 3 had a color that the judges preferred over the other samples. The panelists preferred sample 2 based on the two organoleptic test indicators, taste and smell. Sample 2 showed the least amount of protein and had moderate quantities of antioxidants in comparison to the other two samples. Sample 1 exhibited the highest protein content but the lowest antioxidant level in comparison to the other two samples. Moreover, sample 1 was preferred for its texture by the panelists. The evidence suggests that peanut milk vegan cheese has the capacity to serve as a plant-based replacement to traditional cheese. These findings align with the research conducted by Etudier et al. (2020), which concluded that analog cheese made from peanuts has comparable properties to processed cheese made from milk. Vegan cheese has the potential to be perceived as healthier than cow's milk cheese, but it also has the risk of causing sensitivities to particular plant elements. According to Kovacevic et al. (2024), vegetable ingredients have been selected as substitutes for casein in cow's milk due to their healthier fat content and smoother protein texture. However, vegetable ingredients also have the potential to possess more specific allergenic properties and a distinct taste. Therefore, it is necessary to incorporate certain ingredients to enhance the acceptability of the final product formulation. Research on plant-based functional food products, particularly vegan cheese products, is crucial in the future, especially when considering the incorporation of additional nutrients such as antioxidant enrichment. Particularly, it is beneficial to employ advanced cheese processing techniques in order to reduce the incidence of antioxidant oxidation in the raw materials.

4. Conclusions

Peanut milk vegan cheese with bromelain coagulant from pineapple and the addition of carrot extract as an

antioxidant source had the potential to serve as an alternative to analog cheese created from vegetable ingredients. The results showed that sample 2 with 15% carrot extract was the most favored peanut vegan cheese based on organoleptic tests. The protein value of sample 1, which did not contain carrot extract, was the highest among all samples, including cheese made from cow's milk. Sample 3, which contains 30% of carrot extract, had the highest antioxidant activity compared to the other two samples.

Author Contributions

Conceptualization, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; methodology, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; validation, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; formal analysis, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; investigation, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; data curation, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; writing—original draft preparation, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; writing—review and editing, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; visualization, S.M.A-S.; I.Z.; Z.N.; M.S.R.O.; I.; supervision, I. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability

The data used to support the research findings are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflict of interest

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