

# Katuk Leaves (*Sauropus androgynus*) as a Substitute for ZA Improve the Physical and Organoleptic Properties of Nata De Whey

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## ABSTRACT

Utilization of cheese waste, namely whey, is one alternative to increase the added value of whey by processing it into nata de whey. This study aims to determine the effect of using *Katuk* leaves (*Sauropus androgynus* (L) Merr) as a substitute for ZA (Ammonium Sulfate) on the yield, thickness, color, texture, and organoleptic of *Nata* de whey. This study used 8 liters of whey and *Katuk* leaves (*Sauropus androgynus* (L) Merr) as much as 600 g. The experimental design used in this study, namely the Randomized Block Design (RBD) with four treatments and five repetitions. The treatment in this study was A = 0.5% ZA, *Katuk* leaves (B = 5%, C = 10%, and D = 15%). The observed variables were yield, thickness, color, texture, and organoleptic. The results showed that the treatment differed significantly ( $P < 0.05$ ) in yield, thickness, color, texture, and organoleptic. The best treatment result is treatment B with a yield value of 91.94%, thickness of 7.77 mm, lightness ( $L^*$ ) 59.17, greenness ( $a^*$ ) -4.16, yellowness ( $b^*$ ) 7.52, texture 5.25 N/cm<sup>2</sup>, organoleptic color 3.90, scent 3.58, taste 3.82, texture 3.78.

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## Introduction

Whey is a by-product of cheese processing. Much of this waste is produced during cheese processing, but farmers or processors usually do not utilize this whey, so it can be considered unutilized waste. The processing of whey as a food raw material has begun to be carried out a lot for research, so it is hoped that this will provide opportunities for breeders and entrepreneurs to utilize this whey in new businesses and, at the same time, reduce waste levels that can damage the environment (de Wit, 2001).

So far, whey has been widely developed as a functional food ingredient, such as the use of whey in making edible film products (Juliyarsi et al., 2011), whey fermented drinks such as kefir (Prastujati et al., 2018), fermented whey inoculated with *Pediococcus acidilactici* (Melia et al., 2022) and yogurt (Nurminabari et al., 2018). Even the potential of whey yogurt as a natural ingredient to prevent acne (Rahman et al., 2014). Focusing on the use of whey as a food ingredient, the development of whey as an ingredient for making *Nata* is quite interesting to research, and a lot of research has begun.

*Nata* de whey is defined as a product produced from whey with the addition of *Acetobacter xylinum* bacteria, which can produce biocellulose threads that result in a dense *Nata* structure. Like natural

cellulose in general, *Nata* is very good for human health. *Nata* contains dietary fiber, which is useful in the digestion of food in the small intestine and the absorption of air in the large intestine (Setiaji et al., 2002). Making *Nata* de whey uses whey, a by-product of cheese making, sucrose sugar, ZA (Ammonium Sulfate), vinegar acid, and *Acetobacter xylinum* starter. The use of these ingredients each has different functions, such as sugar as a source of Carbon (C) for bacterial growth, the use of ZA in making *Nata* de whey functions as a source of Nitrogen (N), which is helpful for helps maintain bacterial metabolism and vinegar is used as a form of acidic atmosphere when bacterial breeding process.

ZA is not a food ingredient consumed by humans, so it is unsafe if the amount is too excessive and can be toxic to the body (Silitonga et al., 2018). *Nata* using ammonium sulfate and urea contain high levels of metal contamination, such as Cu, Pb, and Zn, which, if consumed continuously, will harm health (Kholifah, 2010). Therefore, a safer material is needed to replace the function of ammonium sulfate as a nitrogen source.

*Katuk* leaves (*Sauropus androgynus* (L) Merr) are food ingredients and medicines that are rich in benefits and contain high nutrients. Some foods or medicines can be obtained from *Katuk* leaves, and

their potential as a food ingredient is tremendous. One of the contents of *Katuk* leaves is protein; each 100 g of *Katuk* leaves contains 4.8 g of protein (Santoso, 2005). Based on this, using *Katuk* leaves as a substitute for ZA is considered a potential source of N for the growth of *Acetobacter xylinum* so that it can produce *Nata*.

In the previous study, which was about using mung bean sprouts to make *Nata de whey* (Gensika, 2014), the treatments were P0 (no boiled water of mung bean sprouts added) and P1, P2, and P3 (5%, 10%, and 15% boiled water of mung bean sprouts added) with a maximum of 15 days of incubation. The results showed that adding 12.5% peanut shell extract made creamy-white *Nata de coco* that was 0.49 cm thick and had a 42% yield.

Based on this, research is required to determine the effect of substituting *Katuk* leaves for ZA on the yield, viscosity, color, texture, and organoleptic qualities of *Nata de whey*.

## Method

The main ingredients used in this study were 8 liters of whey, 600 g of *Katuk* leaves, 10% *Acetobacter xylinum* starter, 1200 ml of water as a solvent, 10% sucrose sugar, 1% acetic acid, ZA (*Zwavelzure Ammoniak*) food grade 0.5% as control. This study used a randomized block design (RBD) with four treatments, namely the addition of *Katuk* leaf extract at 0%, 5%, 10%, 15%, and five repetitions was carried out. The whey incubation period was 14 days. The research was conducted for 2 months (January-April 2023). This research was conducted at the Animal Products Technology Laboratory, Faculty of Animal Sciences, Universitas Andalas.

## Results

### Yield

After 14 days of incubation, the weight of *Nata de whey* can be determined using a scale. The yield test results are presented in Table 1. The average yield of *Nata de whey* containing various proportions of *Katuk* leaves (*Sauropus androgynus* (L.) Merr) ranged from 70.52 to 91.94 percent for treatments A through D. The highest average yield value was found in the *Katuk* leaf treatment with treatment B, namely 91.94%, but not significantly different ( $P > 0.05$ ) with treatment A, namely 85.28%.

**Table 1.** Average Yield of *Nata de Whey*

| Treatment | Average (%)        |
|-----------|--------------------|
| A         | 85.28 <sup>b</sup> |
| B         | 91.94 <sup>b</sup> |
| C         | 74.10 <sup>a</sup> |
| D         | 70.52 <sup>a</sup> |

Note: Numbers followed by the superscript letter (a,b,c,d) which are different in the treatment, indicate significantly different ( $P < 0.05$ )

DMRT further test results showed that the *Nata de whey* yield in treatment B was not significantly different ( $P > 0.05$ ) from treatment A. Treatment C was significantly different ( $P < 0.05$ ) from treatments

A and B, but not significantly different ( $P > 0.05$ ) from D treatment

### Thickness

*Nata de whey* thickness measurement was carried out after measuring the yield. Thickness was measured using a 0.05 mm caliper in five parts of the *Nata*. The results of measuring the thickness of *Nata de whey* can be seen in Table 2. Table 2 below shows that the average thickness of different *Nata de whey* from treatment A to treatment D ranges between 5.35-7.77 mm. The highest average thickness value was obtained in treatment B with a percentage of 5%, namely 7.77 mm.

**Table 2.** Average Thickness of *Nata de Whey*

| Treatment | Average (mm)      |
|-----------|-------------------|
| A         | 7.18 <sup>b</sup> |
| B         | 7.77 <sup>b</sup> |
| C         | 5.76 <sup>a</sup> |
| D         | 5.35 <sup>a</sup> |

Note: Numbers followed by the superscript letter (a,b,c,d), which are different in the treatment, indicate significantly different ( $P < 0.05$ )

DMRT follow-up test results showed that the thickness of *Nata de whey* in treatment B was not significantly different ( $P < 0.05$ ) from treatment A. Treatment B was significantly different ( $P < 0.05$ ) from treatments C and D. Treatment C was significantly different ( $P < 0.05$ ) from treatments A and B, but not significantly different ( $P > 0.05$ ) from treatment D.

### Color

Color testing with the  $L^*a^*b^*$  system using a colorimeter with the Color Flex EZ brand. The color values obtained will be explained in Table 3.

The average Lightness value of *Nata de whey* can be seen in Table 3. Table 3 below shows that the average lightness ( $L^*$ ) of *Nata de whey* that differed from treatment A to treatment D ranged from 57.72 to 65.06. The highest average lightness value ( $L^*$ ) was found in the 0.5% ZA control treatment, namely 65.06, while the lowest treatment was in treatment C with an average of 57.72 and not significantly different ( $P > 0.05$ ) with treatment D 58.18 and treatment B 59.17.

**Table 3.** Average  $L^*a^*b^*$  Color value in *Nata de whey*

| Treatment | ( $L^*$ )          | ( $a^*$ )          | ( $b^*$ )          |
|-----------|--------------------|--------------------|--------------------|
| A         | 65.06 <sup>b</sup> | -3.18 <sup>b</sup> | 3.58 <sup>a</sup>  |
| B         | 59.17 <sup>a</sup> | -4.16 <sup>a</sup> | 7.52 <sup>b</sup>  |
| C         | 57.72 <sup>a</sup> | -4.26 <sup>a</sup> | 8.18 <sup>b</sup>  |
| D         | 58.18 <sup>a</sup> | -4.38 <sup>a</sup> | 10.34 <sup>c</sup> |

Note: Numbers followed by the superscript letter (a,b,c,d), which are different in the treatment, indicate significantly different ( $P < 0.05$ )

Further, DMRT tests showed that treatment A was significantly different ( $P < 0.05$ ) from treatments B, C, and D.

The average Greenness value of *Nata de whey* can be seen in Table 3. above. The chromatic color of the red-green mixture is indicated by the  $a^*$  value ( $a^+ = 0-100$  for red,  $a^- = 0-(-80)$  for green (Engelen, 2017)). In Table 3. above, it can be seen that the average greenness ( $a^*$ ) of *Nata de whey* which differed from treatment A to treatment D ranged from (-3.18) - (-4.38), with the highest average greenness value ( $a^*$ ) found in treatment A, namely - 3.18, while the lowest treatment average was with the use of *Katuk* leaves (*Sauropus androgynus* (L) Merr). The results of further DMRT tests showed that treatment A was significantly different ( $P < 0.05$ ) from treatments B, C, and D. Treatment B was not significantly different ( $P > 0.05$ ) from treatments C and D but was significantly different ( $P < 0.05$ ) from treatment A.

Furthermore, the average yellowness value of *Nata de whey* can be seen in Table 3. The chromatic color of the blue-yellow mixture is indicated by the  $b^*$  value ( $b^+ = 0 - 70$  for yellow,  $b^- = 0- (-70)$ ) for blue (Engelen, 2017). Table 3. above shows that the average yellowness ( $b^*$ ) of *Nata de whey*, which differed from treatment A to treatment D, ranged from 3.58 to 10.34. The highest ( $b^*$ ) value was found in treatment D, which was 10.34, and the most beautiful treatment was obtained by treatment A, which was 3.58. DMRT further test results showed that treatment A showed significantly different results ( $P < 0.05$ ) from treatments B, C, and D. Treatment B was not significantly different ( $P > 0.05$ ) from treatment C but was significantly different ( $P < 0.05$ ) from treatments A and D. Treatment D was significantly different ( $P < 0.05$ ) from treatments A, B and C.

### Texture

Texture testing was carried out using a Texture Analyzer with the Brookfield brand model CT3. The texture test results can be seen in Table 4. In Table 4 below, it can be seen that the average texture of *Nata de whey* with treatment A to treatment D ranges between 3.81-5.25 N/cm<sup>2</sup>. The highest average texture value was in treatment B, with an average of 5.25 N/cm<sup>2</sup>, and was not significantly different ( $P > 0.05$ ) from treatment C, namely 4.99 N/cm<sup>2</sup>.

**Table 4.** Average Texture of *Nata de Whey*

| Treatment | Average (N/cm <sup>2</sup> ) |
|-----------|------------------------------|
| A         | 3.81 <sup>a</sup>            |
| B         | 5.25 <sup>c</sup>            |
| C         | 4.99 <sup>bc</sup>           |
| D         | 4.27 <sup>ab</sup>           |

Note: Numbers followed by the superscript letter (a,b,c,d), which are different in the treatment, indicate significantly different ( $P < 0.05$ )

The results of further DMRT tests showed that treatment A was significantly different ( $P < 0.05$ )

from treatments B and C but not significantly different ( $P > 0.05$ ) from treatment D. Treatment B was not significantly different ( $P > 0.05$ ) from treatment C but was significantly different with treatment A and D.

### Organoleptic

After carrying out the organoleptic test by the panelists, the measurement results were obtained, which can be seen in Table 5.

**Table 5.** Mean Organoleptic Color Values of *Nata de Whey*

| Treatment | Color             | Scent              | Taste             | Texture |
|-----------|-------------------|--------------------|-------------------|---------|
| A         | 4.82 <sup>c</sup> | 3.78 <sup>ab</sup> | 3.72 <sup>a</sup> | 3.90    |
| B         | 3.86 <sup>b</sup> | 3.58 <sup>a</sup>  | 3.82 <sup>a</sup> | 3.78    |
| C         | 3.82 <sup>b</sup> | 4.12 <sup>b</sup>  | 4.20 <sup>b</sup> | 3.94    |
| D         | 3.36 <sup>a</sup> | 3.42 <sup>a</sup>  | 3.88 <sup>a</sup> | 3.80    |

Note: Numbers followed by the superscript letter (a,b,c,d), which are different in the treatment, indicate significantly different ( $P < 0.05$ )

Table 5 above shows that the average color of the different *Nata de whey* from treatment A to treatment D ranged from 3.36–4.82. The highest mean color value was in treatment A, namely 4.82, which means really like it, while the lowest was in treatment D, with a mean of 3.4, namely neutral.

Based on further DMRT organoleptic color tests, treatment A showed significantly different results ( $P < 0.05$ ) from treatments B, C, and D. Treatment B was not significantly different ( $P > 0.05$ ) from treatment C but was significantly different ( $P < 0.05$ ) from treatment A and D. Treatment D was significantly different ( $P < 0.05$ ) from treatment A, B, and treatment C.

The result of the scent can be seen in Table 5. it can be seen that the average scent of *Nata de whey*, which differed from treatment A to treatment D, ranged from 3.42–4.12. Adding *Katuk* leaves has a significant effect ( $P < 0.05$ ) on the organoleptic value of *Nata de whey* scent. The highest mean scent value was in treatment C, namely 4.12, which means like, while the lowest was in treatment D, with a mean of 3.42, which means neutral. According to further DMRT organoleptic scent tests, treatment A showed results that were not significantly different ( $P < 0.05$ ) from treatments B, C, and D. Treatment B was not significantly different ( $P > 0.05$ ) from treatments A and D but was significantly different ( $P < 0.05$ ) with treatment C.

Furthermore, the organoleptic taste test results can be seen in Table 5. In Table 5. above, it can be seen that the average taste of *Nata de whey*, which differs from treatment A to treatment D, ranges between 3.72 and 4.20. The highest mean taste score was in treatment C, namely 4.20, which means like it, while the lowest was in treatment A with a mean of 3.72. Based on further DMRT tests, treatments B and D were not significantly different ( $P > 0.05$ ) from treatment A but significantly different ( $P < 0.05$ ) from treatment C.

Moreover, the scent organoleptic test results can be seen in Table 5. In Table 5. above, it can be seen that the average texture of *Nata* de whey with different percentages of *Katuk* leaves (*Sauropus androgynus* (L) Merr) from treatment A to treatment D ranged from 3.80–3.90, which means the panelists liked the texture of the *Nata*. Based on the statistical analysis results, tests showed that adding *Katuk* leaves had no significant effect ( $P > 0.05$ ) on the organoleptic value of the *Nata* de whey texture.

## Discussion

The highest average yield value was obtained in treatment B. Namely, adding *Katuk* leaves (*Sauropus androgynus* (L) Merr) at a percentage of 5% would decrease along with the percentage of *Katuk* leaves added. Optimal B treatment meets the need to increase the yield of *Nata* de whey. This is because treatment B is sufficient for nutritional needs, especially nitrogen, so it can increase the activity of *Acetobacter xylinum* bacteria for metabolism so that the results of this metabolism in the form of biocellulose produced increase. This statement is following the opinion of Ernawati (2012), namely that the treatment of adding 5% soybean sprout extract gets the highest yield value, adding an organic nitrogen source such as 5% soybean sprout extract to the fermentation medium is the best treatment to get the highest yield. According to Hastuti (2015), the addition of weight and thickness of *Nata* is affected by the increased activity of *Acetobacter xylinum* bacteria. These bacteria can to synthesize extracellular cellulose to form *Nata* pellicle on the surface of the fermentation medium. So that in line with the addition of thickness, the weight of the *Nata* also increases. Based on Kuncara's research (2017), the use of mung bean sprout extract even though it has an insignificant effect on the physical characteristics of *nata* de soya, but qualitatively it can be used to replace ZA as a nitrogen source. According to Safitri et al. (2017) the nitrogen content contained in soy bean sprouts is the optimum amount for bacterial activity, but not higher than the nitrogen content contained in urea.

The highest average thickness value was obtained in treatment B, which was 7.77 mm. Based on this research, it can be seen that optimal use of nitrogen sources can increase the thickness of *Nata*; nitrogen can help the metabolic process of *Acetobacter xylinum* bacteria, thereby accelerating the synthesis of sugar into cellulose increasing the thickness of the *Nata* obtained. These results are following Ernawati's (2012) research. The addition of 5% green bean sprout extract optimally increased the thickness of *Nata* de milko, namely 1.52 cm, and the highest thickness of *Nata* was seen in the treatment with the addition of 5% soybean sprout extract, 1.69 cm. According to Ningsih et al. (2021), an increase in the thickness of *Nata* can occur due to the availability of adequate nitrogen sources.

The thickness of *Nata* de whey began to decrease in treatment C, which was 5.76 mm. This is because the use of a percentage of *Katuk* leaves that is too high inhibits the process of forming cellulose. After

all, the nitrogen content in *Katuk* leaves, which is too high can reduce the work of *Acetobacter xylinum* bacteria in synthesizing cellulose, which forms the *Nata* layer so that the thickness of the *Nata* is reduced.

*Nata* de whey, with the highest average brightness value, was obtained by treatment A, which was equal to 65.06 and was significantly different ( $P < 0.05$ ) from the addition of *Katuk* leaf extract (*Sauropus androgynus* (L) Merr). This is because *Katuk* leaves have chlorophyll and xanthophyll pigments, which can reduce the brightness of *Nata* de whey. From the results of the brightness obtained, it can be seen that the use of different nitrogen sources affects the brightness level because the content in natural nitrogen sources contains different compositions, such as *Katuk* leaves containing chlorophyll and xanthophyll, which are leaf pigments that function as plant photosynthesis which tend to be green in color, these pigments lower the brightness on *Nata* de whey. This study follows the results of Djayasaputra's research (2017) that the color parameter ( $L^*$ ) for *Nata* with an additional nitrogen source of mung bean sprout extract was significantly different ( $P < 0.05$ ) from that of instant yeast, where the value ( $L^*$ ) for *Nata* with yeast nitrogen source is higher which means it has a higher brightness level than *Nata* with mung bean sprout extract.

Greenness results ( $a^*$ ) with the highest value were obtained by treatment A with an average of -3.18, which showed a greenness value that was smaller than the treatment using *Katuk* leaves (*Sauropus androgynus* (L) Merr). The average greenness value ( $a^*$ ) with the use of *Katuk* leaves shows a number with a negative value along with the more significant addition of *Katuk* leaves, which shows a greater greenness value. These results are following Djayasaputra's research (2017), namely the production of *Nata* de waluh using mung bean sprouts and yeast, showing that the color parameter ( $a^*$ ) obtained a negative value for all treatments. *Nata* de whey, with the lowest ( $a^*$ ) value was obtained by the addition of *Katuk* leaves (*Sauropus androgynus* (L) Merr). This is because the green pigment is present in *Katuk* leaves (*Sauropus androgynus* (L) Merr). Namely, chlorophyll pigment can affect the color of *Nata* to become greener as more *Katuk* leaves (*Sauropus androgynus* (L) Merr) are added to *Nata* de whey.

*Nata* de whey with more yellow color is due to the yellow pigment content in the ingredients for making *Nata* de whey, as the whey contains the yellow riboflavin pigment. This is the opinion of Widuri (2018), which states that riboflavin gives a yellowish color effect to whey. Riboflavin is heat resistant and can affect the value of the yellow color in the product. In addition, *Katuk* leaves also have carotene and xanthophyll, which are yellow pigments in *Katuk* leaves.

The highest average texture value was obtained in treatment B, namely 5.25 N/cm<sup>2</sup>. This is because the texture is affected by the yield and thickness. After all, *Nata* is a type of fiber which, if you experience an increase in weight and thickness, the texture will also increase. According to the opinion of Nisa et al.

(2001) the texture of nata (soft) indicates more insoluble fibers are formed. The hardness of the texture is closely related to the density of the cellulose network. This follows the opinion of Ernawati (2012) that thickness and fiber content affect the texture of the resulting *Nata*. The average texture value shows the difference in texture value on ZA sources and *Katuk* leaves (*Sauropus androgynus* (L) Merr). The texture value with ZA treatment is lower than using *Katuk* leaves. This is because in ZA, the fiber content formed is lower, but the water content is higher. High fiber content will increase the textural value. In contrast, the high water content value in *Nata* will produce *Nata* texture, which is not chewy because water is trapped in the *Nata* fibers and makes *Nata* harder.

The organoleptic difference in the color of *Nata* de whey with the addition of *Katuk* leaves is because the *Katuk* leaves used in the study have a darker color than the treatment using ZA. After all, *Katuk* leaves contain chlorophyll which is green in color, so this pigment can be trapped in the *Nata* fiber and produces a green color. darker in the panelists' judgment. This is following the opinion of Ningsih et al. (2021), the color of this medium will be trapped in the transparent fiber structure of the *Nata*, and the resulting color from using mung bean sprouts is darker than using ZA in making *Nata*.

The results of the organoleptic test on the scent of *Nata* de whey with treatments B and C were not significantly different ( $P > 0.05$ ) from treatment A, namely the value of liking scent. While the lowest treatment was obtained by treatment D with a neutral scent value. The scent of *Nata*, according to the standard, is odorless or average, but the scent of *Nata* can be affected by the whey scent that remains even after soaking and boiling.

The best taste in the organoleptic taste test was obtained in treatment C, adding 10% *Katuk* (*Sauropus androgynus* (L) Merr) leaves with a value of 4.20, meaning the panelists liked the product. The standard taste of *Nata* is bland, but there is a sweet taste to *Nata*, which is caused when 10% sugar is added during boiling so that the *Nata* tastes slightly sweet.

The addition of *Katuk* leaves did not affect the results of the organoleptic texture, with an average value ranging from 3.78-3.94 with an organoleptic value of like. This is because *Nata*, which has undergone boiling, will produce a chewy texture and is dense due to cellulose's density.

## Conclusion

The effect of using *Katuk* leaves (*Sauropus androgynus* (L) Merr) in making *Nata* de whey was significantly different  $P (< 0.05)$  in yield, thickness, color, texture, color organoleptic, scent organoleptic, taste organoleptic but not significantly different ( $P > 0.05$ ) on organoleptic texture. The use of *Katuk* leaves (*Sauropus androgynus* (L) Merr) in making *Nata* de whey treatment B is the best treatment with a yield value of 91.94%, thickness 7.77 mm, color ( $L^*$ ) 59.17, color ( $a^*$ ) -4.162, color ( $b^*$ ) 7.52, texture 5.25 N/cm<sup>2</sup>, color organoleptic 3.90, scent

organoleptic 3.58, taste organoleptic 3.82, texture organoleptic 3.78. The addition of *katuk* leaves in the manufacture of *nata* de whey can be used as an alternative to natural ingredients as a source of nitrogen to replace ZA.

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## Author Contribution and Competing Interest

FVA: Conducted the investigation and wrote the manuscript. The data was processed by SM, IJ, and FVA. The final manuscript was read and approved by all writers.

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