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# THE SUBSTITUTION OF LEMURU FISH WASTE IN THE STUDY OF MAKING NATA DE FISH

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**ABSTRACT**

Nata is the result of fermentation products by *Acetobacter xylinum*. If nata de coco is often found with coconut water media, then nata de fish is made with unused lemuru stew media and has the potential to be waste because it results in polluting the environment. This study aims to find out how to make nata by comparing the substitution of lemuru decoction to the quality of nata de fish. Experimental design used 2x2 factorial RAL, namely the use of clean water 1.5 L and 2 L, analysis of each parameter including, the nata thickness parameter in each treatment is P<0.01, the yield parameter in each treatment is P>0.01, the wet weight parameter is P>0.01, the protein content parameter is P>0.01, while the Escherichia coli parameter is <3 MPN/gr

Keywords: Substitution, Waste, Lemuru, Nata De Fish

## I. INTRODUCTION

Banyuwangi is a coastal area rich in natural resources. Among these resources, lemuru fish reigns supreme as a marine commodity, as it remains abundant to this day. During times of abundance, the local population processes lemuru fish into long-lasting products like pindang lemuru, lemuru flour, shrimp paste, and petis.

The process of creating these products results in lemuru decoction, which is unfortunately discarded as waste. However, lemuru decoction still contains valuable nutrients like protein, fat, and omega 3, essential for brain and heart development. Therefore, this discarded resource presents an opportunity to create additional value for the local industry by repurposing it into processed fish products like nata de fish.

Nata is a gel-shaped food with a chewy, dense, white, and slightly transparent texture. Nata is usually used as dessert as well as as canned food mixed with fresh fruits. The manufacture of nata can take advantage of a variety of raw materials, such as fruit juice and vegetables as long as the ingredients correspond to the bacterial growth medium. Such as nata de coco (Nurdyansyah & Widyastuti, 2017), nata de soya (Putri & Fatimah, 2021), nata de banana (Suarti, *et al.,*2013), nata de aloe (Susatyo & Nurhayati, 2013), while Nata de fish is a nata made from the fermentation of *Acetobacter xylinum* with the main basic ingredient of fish decoction or

lemuru fish stew which is classified as waste (Adharani *et al.,*2017).

The selection of raw materials is based on the factors of ease of obtaining raw materials, the availability of raw materials, the nature of fermentation, and the price of raw materials. Variations in the use of raw materials in nata making are carried out to utilize materials that are not optimal even though they can have added value, overcome seasonal raw materials, and create variations in nata products. The abundance of lemuru fish in Banyuwangi is a raw material that is easy to get and is cheap to be used as nata de fish. so that it becomes something more useful. This study was conducted referring to adharani *et al.,*(2017) which was modified, the modified factors were the manufacture of lemuru fish decoction and the substitution of lemuru fish decoction added in the growth medium of *A. xylinum*.

## II. RESEARCH METHODS

### 2.1 Time and Place of Research

This research was conducted in July-August 2023. Tool and material preparation, nata de fish media fermentation, and physical quality testing were conducted at the Fishery Product Technology Laboratory, PGRI University Banyuwangi. Nata de fish proximate testing was conducted at the Jember State Polytechnic Bioscience Laboratory.

### 2.2 Materials and tools

The main ingredients in making nata de fish are lemuru fish stew or lemuru stew, *Acetobacter xylinum* bacteria, vinegar, sugar, food grade ZA (zwavelzure ammonium), alcohol, and tissue. The tools used in the study were rectangular plastic containers measuring (P (34cm), L (24cm), T (1.8 cm)), fermentation incubators, brown paper, pans, analytical scales, pH meters. The use of tools in protein analysis and carbohydrate analysis with the Indonesian National Standard method (SNI.01-2354. 4-2006 and SNI ISO 11292: 2015). To measure the thickness of nata using calipers and plastic, while organoleptic measurements are carried out visually.

### 2.3 Research methods

Here is the process of making nata de fsih:

**1. Making Lemuru Fish Stew:**

1. Wash 1 Kg of lemuru fish, then remove the gills and head
2. Boil lemuru fish with water as much as 1000 ml (A1) and 1500 ml (A2) for 30-45 minutes
3. After boiling, remove the fish and strain the boiled water using a colander covered with a filter cloth
4. Lemuru fish stew is ready for use, and can be stored in the refrigerator as stock in the manufacture of nata de fish.

**2. Preparation for Fermentation:**

1. Gather all ingredients for the fermentation medium, including decoction of lemuru at each different treatment (P1: 15ml of lemuru boiled water, P2: 50ml of lemuru boiled water, P3: 75ml of lemuru boiled water, P4: 100ml of lemuru boiled water, P5: 125ml of lemuru boiled water), 1 L of clean water, 200 gr of sugar, 0.5 gr of *food grade* ZA, 25 ml of vinegar, and 15% of *Acetobacter xylinum* bacteria.
2. All ingredients except *A. xylinum* bring to a boil
3. Pour the substrate into a plastic tray that has been cleaned using alcohol

and then let it cool (*A. xylinum* will die when combined with a hot medium), then put 15% *A. xylinum* and homogenize it by shaking the tray gently until evenly distributed.

4. Close the tray using brown paper and rubber binder on the edges
5. The medium is fermented in a fermentation incubator with a temperature of 27ºC for 15 days.

**3. Paramater Measurement:**

1. **Thickness.** Nata thickness was measured using calipers in all five treatments (P1; P2; P3; P4; P5) and both factors (A1 and A2). The reported value is the average of each treatment's measurements.
2. **Rendement (Yield).** The nata yield is obtained by, the formed nata is dried for 30 minutes (to reduce water dripping from the nata) and weighed to determine the wet weight, then the calculation of changes is carried out using the formula (Goh *et al.,* 2012)

$$\text{Rendemen (\%)} = \frac{Berat\ basah\ nata\ (\frac{g}{L})}{Volumen\ substrat\ (mL)} \text{ x } 100\%$$

3. **Protein Test.** Protein testing using the Indonesian National Standard (SNI) method 01-2354.4-2006. The average of each treatment's test results.
4. **Carbohydrate Test.**
   Carbohydrate testing using the Indonesian National Standard (SNI)-The International Organization fo Standardization (ISO) method 11292:2015. The average of each treatment's test results.
5. **Organoleptic Test (Color, Texture, Aroma)**
   Organoleptic assessment is performed for the evaluation of color, texture, and aroma using a hedonic test (favorability) with numerical values on a hedonic scale as shown in the following table 1.

**Table 1.** Numerical Value of Organoleptic Test

| Color aspect | Score | Texture aspect | Score | Aroma aspect | Score |
|---|---|---|---|---|---|
| Putih jernih | 4 | Padat | 4 | Sangat asam | 4 |
| Putih | 3 | Sangat kenyal | 3 | Asam | 3 |
| Putih kekuningan | 2 | Kenyal | 2 | Agak asam | 2 |
| Kuning | 1 | Tidak kenyal | 1 | Tidak asam | 1 |

**2.4 Research Design**

This study, based on the design of Adharani *et al.,* (2017), employs a Completely Randomized Design (RAL) with two factors:

- Factor 1: Water concentration (A) at two levels:
  - A1 = 1000 ml
  - A2 = 1500 ml
- Factor 2: Lemuru boiled water (P) at five levels:

- P1 = 15 ml
- P2 = 50 ml
- P3 = 75 ml
- P4 = 100 ml
- P5 = 125 ml

The design results in 2 x 5 = 10 treatment combinations, each replicated four times (n = 40).

### 2.5 Data Analysis

The data observed in the calculation of the thickness and yield of nata de fish were analyzed using two-way ANOVA. Data analysis continued with the Duncan Test at a confidence level of 95% if the results of the fingerprints showed a noticeable difference (for pairwise comparisons of significant mean differences). Protein, carbohydrate, and organoleptic data analyzed with ANOVA, reported as a percentage.

## III. RESULTS AND DISCUSSION

### 3.1 Production of Nata de fish

The substitution of lemuru decoction to the fermentation of nata de fish carried out for 14 days is characterized by the formation of a layer of nata on the surface of the medium, here is the resulting nata de fish:

**Figure 1.** Nata De Fish Productions

### 3.2 Nata Thickness

The maximum thickness of nata de fish produced from the substitution of lemuru decoction is obtained after a fermentation process for 15 days. It can be seen in the image below:

**Table 2.** Average Thickness of Nata de Fish in Each Treatment

| Treatment | Average (cm) | Notation |
|---|---|---|
| A1P1 (15ml) | 2.3 | a |
| A1P2 (50ml) | 1.98 | a |
| A1P3 (75ml) | 1.83 | ab |
| A1P4 (100ml) | 1.28 | b |
| A1P5 (125ml) | 1.28 | bc |
| A2P1 (15ml) | 2.0 | a |
| A2P2 (50ml) | 1.8 | a |
| A2P3 (75ml) | 1.58 | a |
| A2P4 (100ml) | 1.35 | a |
| A2P5 (125ml) | 0.93 | ab |

It can be seen in Table. 2 above that the highest nata thikness was obtained in the A1P1 treatment of 2.3 cm while the lowest in the A2P5 treatment was 0.93 cm. Be more specific about what kind of analysis and what parameters were analyzed, it can be seen that the factor of providing A in both 1000 ml and 1500 ml for B to the substitution of lemuru decoction used has no noticeable effect on the thickness of nata de fish. The less lower consentrations of lemuru boiled water is given to each factor A, resulting in greater thickness, and vice versa. This is because the substitution of lemuru decoction given contains protein compounds, so that *A. xylinum* bacteria do not denature or change the structure of protein molecules in the fermentation process (Basamalah *et al.,*2018).

Sources of nutrients that are needed by *A. xylinum* and affect the inhibition of nata in the fermentation process include carbon sources, nitorgen, and pH. The source of carbon used in the fermentation of nata is carbohydrates, which are classified as monosaccharides and disaccharides. Media containing glucose, sucrose, and lactose can help the formation of nata. The most widely used sugar medium is sucrose or granulated sugar. Granulated sugar can also function as an inducer material that plays a role in the formation of the polymerase extracellular enzyme which works to compile nata threads so that the formation of nata can be maximized. The use of lemuru decoction as a substitute for nata fermentation media obtained a maximum thickness of 2.3 cm, these results were better than several other juice uses such as banana peel juice of 2.1 cm (Suarti *et al.,*2013), seaweed essence of 0.41 cm (Racmawati *et al.,*2017), cassava juice of 1.37 cm (Putriana and Aminah, 2013), saru tahu (soya) of 1.69 cm (Yanti *et al.,* 2020).

### 3.3 Amendments

The resulting amendment is the percentage of the product produced from the substrate of the nata de fish fermentation medium. The percentage of amendments can be seen in the table 3 below.

Based on Table 3. above shows that the highest percentage of yield was obtained in the A1P1 treatment of 92% while the lowest in the A2P5 treatment was 20%. Based on the results of statistical analysis of fingerprints, it can be seen that the factor of giving water both 1000 ml and 1500 ml (A1 and A2) to the substitution of lemuru decoction used has no real effect on the amendment of nata de fish. This is in line with the nata thickness test obtained that, The less lower consentrations of lemuru boiled water given to each factor A, results in a greater percentage of the yield and vice versa.

**Table 3.** Average Nata de Fish Amendments In Each Treatment

| Treatment | Average (%) | Notation |
|---|---|---|
| A1P1 (15ml) | 92 | a |
| A1P2 (50ml) | 77 | a |
| A1P3 (75ml) | 72 | a |
| A1P4 (100ml) | 65 | ab |
| A1P5 (125ml) | 38 | b |
| A2P1 (15ml) | 84 | a |
| A2P2 (50ml) | 63 | a |
| A2P3 (75ml) | 44 | b |
| A2P4 (100ml) | 55 | ab |
| A2P5 (125ml) | 20 | c |

The yield is influenced by substrate variations, material composition, environmental conditions, and the ability of *A. xylinum* to produce cellulose (Putri *et al.,*2021). The maximum nata thickness produced at the A1P1 treatment (Table 2.), resulted in a maximum yield also in A1P1 (Suarti *et al.,*2013). This is also supported by the container used in the fermentation process, that the amendment and invulnerability of nata are not only obtained by the media factor but the container used. In containers with a wide surface, the growth of nata will be faster so that the yield of nata will be greater than using containers that have a narrow surface. This is caused by a container whose surface is wide, it has good air circulation so that it can help the fermentation process run well because *A. xylinum* will grow optimally (Syukroni *et al.,*2013).

### 3.4 Protein Levels

Protein is a group of macronutrients that are the building and regulating agents of the body. Protein can provide nutrients for daily consumption. The result of the protein content contained in nata de fish after fermentation for 15 days, as follows :

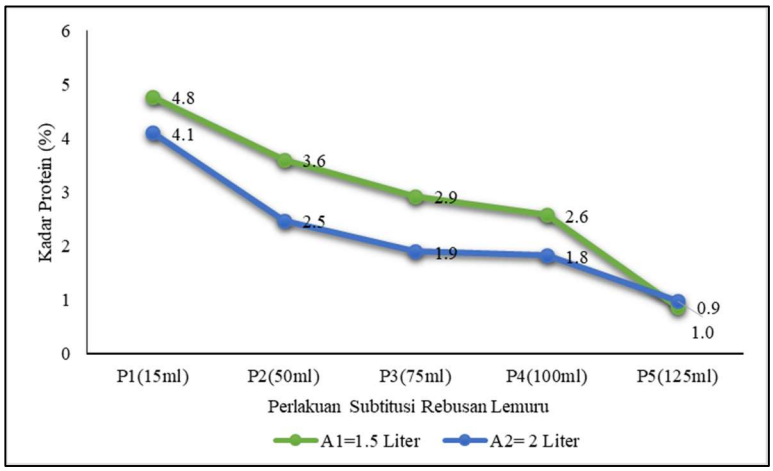


**Figure 2.** Nata De Fish Protein Content Of Each Treatment

Based on Figure 2. above shows that the highest percentage of protein content was obtained in the A1P1 treatment of 4.8% and in A2P1 of 4.1% while the lowest protein content in the A1P5 treatment was 0.9% and A2P5 was 1%. Based on the results of statistical analysis of fingerprints, it can be seen that the factor of giving water both 1000 ml and 1500 ml (A1 and A2) to the substitution of lemuru decoction used has no noticeable effect on the protein content of nata de fish. This shows that the less lower consentrations of lemuru boiled water given to each factor A, resulting in a greater percentage of protein and vice versa.

The addition of ZA food grade affects the level of protein formed in nata along with increasing the cell mass of *A. xylinum* organisms that grow during fermentation, so as to be able to add protein levels produced (Iskandar, 2010). The protein content contained in lemuru boiled water is 44.5% per 50 ml (Adharani *et al.,* 2017) so that it affects the protein levels in the nata produced. In addition, the presence of other elements such as P (phosphorus) and S (sulfur) in nitrogen sources will have a major influence on protein levels because these elements play a role in the formation and proliferation of bacterial cells that affect the amount of cellulose formed (Tsalagkas, 2015).

### 3.5 Carbohydrate Levels

Carbohydrates are an important nutrient in nata that functions as a source of energy. The results of the carbohydrate analysis are as follows :

Based on Figure 3. above shows that the highest percentage of carbohydrate content was obtained in the A1P1 treatment of 8.2% and in A2P1 of 7.2% while the lowest carbohydrate content in the A2P5 treatment was 2.9% and A1P5 was 4.2%. Based on the results of statistical analysis of fingerprints, it can be seen that the factor of giving water both 1000 ml and 1500 ml (A1 and A2) to the

substitution of lemuru decoction used has no noticeable effect on the protein content of nata de fish. This shows that the less low consentrations of lemuru boiled water given to each factor A, resulting in a greater percentage of carbohydrates and vice versa.

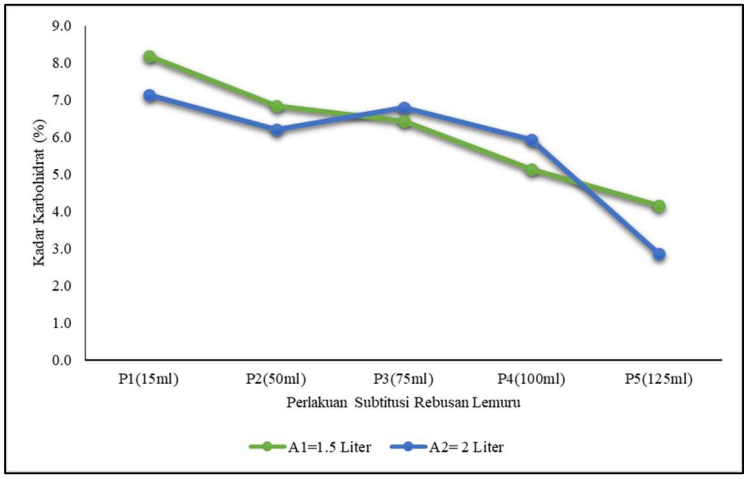


**Figure 3.** Carbohydrate Levels of Nata De Fish Each Treatment

Carbohydrates contained in nata are caused by the presence of sugar as a source of carbohydrates in the manufacture of nata, where glucose is taken from sugar water in the fermentation medium in forming nata. Such glucose combined with fatty acids forms a precursor (nata layer). The precursors are secreted along with enzymes that will convert glucose into cellulose outside the cell, so that the more cellulose that is formed will result in the carbohydrate levels also increasing. *A. xylinum* can synthesize some sugars into cellulose and some of them decompose into acetic acid which will lower the pH of the medium.

### 3.6 Organoleptic Test

Organoleptic tests using sensory tools include color (sight), texture (touch) and aroma (disambiguation). This test is faster and cheaper, but compared to chemical and physical methods, organoleptic tests are strongly influenced by the ability, sensitivity and experience of the panelists (Raharjo, 2018). Organoleptic tests are carried out by scoring the parameters of texture, color, aroma and level of liking of the panelists based on a hedonic scale table, here are the organoleptic results.

The results of the preference test for color in each treatment in each factor (A1 and A2) are white and yellowish white, likely due to the administration of a turbid lemuru decoction and browned butek as the main fermentation medium (Putri *et al.,* 2021). In accordance with the statement from Putriana and Aminah (2013) that a good texture for nata is chewy, dense, and not hard. Putri *et al.,* (2021) added that nata with raw materials other than using coconut water will produce nata with a chewy texture if the factors affecting the fermentation process are well fulfilled.

**Table 4.** Favorite Test Results (Texture, Color, Aroma)

| Perlakuan | Uji Hedonik Tekstur | | Uji Hedonik Warna | | Uji Hedonik Aroma | |
|---|---|---|---|---|---|---|
| | A1=1.5 Liter | A2= 2 Liter | A1=1.5 Liter | A2= 2 Liter | A1=1.5 Liter | A2= 2 Liter |
| P1(15ml) | Sangat kenyal | Padat | Putih jernih | Putih jernih | Sangat asam | Sangat asam |
| P2(50ml) | Sangat kenyal | Sangat kenyal | Putih | Putih | Asam | Asam |
| P3(75ml) | Kenyal | Kenyal | Putih kekuningan | Putih kekuningan | Asam | Agak asam |
| P4(100ml) | Kenyal | Kenyal | Putih kekuningan | Putih kekuningan | Agak asam | Agak asam |
| P5(125ml) | Kenyal | Kenyal | Putih kekuningan | Putih kekuningan | Agak asam | Agak asam |

The results of the preference test for color in each treatment in each factor (A1 and A2) are white and yellowish white, this is possible because the administration of a browned butek and browned butek is the main medium in the fermentation of Putri *et al.,* (2021). According to Negara et al. (2016) color is the first sensory that can be seen directly by consumers or panelists. The color of nata is influenced by the amount of *A. xylinum* used because it affects the color and thickness of nata, while the thickness of nata will affect the color produced. The thicker the nata, the more cloudy or unclear the color will be.

The results of the liking test for aromas in each treatment at each factor (A1 and A2) are acidic and slightly acidic. Aroma is one of the important factors in determining the quality of a food ingredient because consumer preferences are influenced by the aroma of the product. Almost all nata products from various raw materials produce a sour aroma. The sour aroma contained in nata is caused by the activity of microorganisms during fermentation which produces by-products in the form of acid compounds Putri *et al.,* (2021).

## IV. CONCLUSION

This study investigated the impact of clean water delivery (1.5 L and 2 L) on various parameters of nata de fish production. The results revealed significant interactions between water delivery and thickness ($p < 0.01$), amendment ($p < 0.01$), and protein levels ($p > 0.01$), suggesting that the amount of clean water used significantly influences these aspects of nata de fish. Higher water delivery led to thicker nata with higher protein content, but a smaller effect was observed on amendment. All treatments showed low Escherichia coli presence (< 3 MPN/g), indicating safe nata de fish production regardless of water delivery amount. However, further research with larger sample sizes is needed to confirm these findings and explore the underlying mechanisms behind the water delivery-parameter interactions.

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