The effect of starter fermentation time and the different volumes of starters in making nata de arenga

Ira Taskirawati1, Surmi1, Baharuddin1 and Rahmat Safe'i2

1Faculty of Forestry, Hasanuddin University, JI Perintis Kemerdekaan Km. 10 Makassar, Indonesia 90245
2Department of Forestry, Faculty of Agriculture, University of Lampung, Jln. Prof. Dr. Ir. Soemantri Brojonegoro No. 1, Gedong Meneng, Bandar Lampung, 35145, Indonesia

E-mail: tasqira@unhas.ac.id

Abstract. Nira derived from palm sugar (Arenga pinnata Merr.) can be made into several processed products such as brown sugar, vinegar, and wine. Nata de arenga is one of the products that utilizing from nira of palm sugar as one of the basic materials. This study aims to determine the Effect of starter fermentation time and the difference in the volume of starters for making nata from nira of Arenga pinnata. There are two stages used in this study; the first is making the starter, and the second is making nata. In the making of a starter, there are three starter fermentation's time, namely starter which fermentation for four days (F1), six days (F2), and eight days (F3). In making nata, given the three different variations volume of starter, i.e., 100 ml (Vs), 200 ml (Vb), and 300 ml (Vc) combined with starter fermentation's time. Each treatment combination is three repetitions to enhance the validity of the results. The research data were then analysed using a completely randomized design (CRD) with a factorial experiment. The results showed the highest average yield in the combination of starter treatment which fermented for eight days with a 300 ml volume of starter (F3Vc). In this combination, the microbe in the fermentation medium are in optimum physiological conditions for the fermentation process.

1. Introduction
Non-timber forest products (NTFPs) are biological forest products, both vegetable, and animal, and their derivative and cultivation products, except for wood originating from forests [1]. Many non-timber forest products use, but only five product development priorities of non-timber forest products were rattan, bamboo, honey, silk, and agarwood [2]. In addition to these five products, palm sugar is another NTFP product that can develop into superior products. Palm sugar plantations are spread throughout Indonesia, especially in South Sulawesi, according to Plantation statistical data in 2006, which has an area of 4.520 ha of sugar palm [3].

One part of the palm sugar that does widely use by the community is the flower. In this palm sugar's flower, nira is produced, which has economic value compared to other products that can be a product from palm sugar. Palm sugar can produce 15-25 liters of nira per day [4]. The nira of palm sugar products do classify into two types; first, products that do not undergo fermentation, for example, brown sugar, and second, products that undergo fermentation such as vinegar, alcohol (wine), and nata.

Nata has a gel-like or gelatinous form. Nata does usually use as a mixture in the manufacture of beverages. Nata is a low-energy food that is good for dietary purposes because the nutritional value of nata is meager [5]. Nata also contains fiber which can facilitate digestion. As a fibrous food, nata
contains 2.5% cellulose, 2.75% crude fiber, 1.5% – 2.8% protein, 0.35% fat and the remaining 95% is water.

Many studies do make nata from various materials, such as cassava (*nata de cassava*) [6], banana (*nata de banana skin*) [7], tofu (*nata de soya*) [8], and cucumber (*nata de cucumber*) [9]. However, in this study, nira from palm sugar was used as the main ingredient in making nata. In addition, the study aimed to determine the effect of starter fermentation time and the difference in the volume of starters for making nata from nira of *Arenga pinnata*.

2. Method
There are two stages in making nata: making mother liquor (Starter), and the second stage is making nata. Mother liquor (starter) is a nira fermented in bottles after being inoculated with *Acetobacter xylinum*; this starter will apply as seeds in making nata.

2.1. Making starter
There are three starter fermentation's time, namely starter which fermentation for four days (F₁), six days (F₂), and eight days (F₃). The steps in making the starter are as follows: two liters of nira are filtered and then put into a pan. After that, add 25 g of bean sprouts per one liter of nira. The nira and bean sprouts cook while stir, then adds 10 ml/liter of vinegar. Stir the solution until it boils; after boiling, turn off the stove. Strain the nira into a prepared container; prepare a sterilized bottle while waiting for the solution not to get too hot. Pour the solution into the 400 ml/bottle, cover the bottle with newsprint, and tie it with a rubber band. Refrigerate this sample for 6 hours. After cooling, open the bottle cap and add 50 ml/bottle of *A. xylinum*. Cover the bottle with newspaper and tie it with a rubber band. Store the starter solution for four days. This method is also used in starter fermentation for six days and eight days; the difference lies in the length of storage of the solution.

2.2. Making *Nata de arenga*
Making nata from nira of palm sugar is done with the following procedure: strain 6 liters of nira into a pan, then add 25 g of bean sprouts per liter of nira. The next step is to cook the nira and bean sprouts while continuing to stir. Add 10 ml of vinegar per liter of nira. This solution does continuously mix until it boils. The hot solution is filtered into a measuring cup and then poured into a container that has been sterilized according to the treatment made (400 ml, 500 ml, 600 ml). Then cover the container with newspaper and tie it with raffia. This solution was cooled for 4 hours. When the solution has cooled, open the container's lid and add 100 ml of starter to the 600 ml container, 200 ml are added to the 500 ml container, and 300 ml are added to the 400 ml container. Cover the container again with newsprint and tie it with a rubber band to maintain the quality, and let it rest for ten days. The treatment in making nata de arenga is shown in Table 1. Each treatment was made three times.

| Table 1. Treatment in making nata de arenga |
|-------------------------------------------|
| Stater Fermentation Time (days) [F]  | Volume Stater (ml) [V]           |
|                                     | 100(a) | 200(b) | 300(c) |
| 4(1)                  | F₁Va  | F₁Vb  | F₁Vc  |
| 6(2)                  | F₂Va  | F₂Vb  | F₂Vc  |
| 8(3)                  | F₃Va  | F₃Vb  | F₃Vc  |

2.3. Variable observed
Observations made were the yield of nata produced. How to calculate the yield of nata processing is as follows:

\[
\text{Yield} = \frac{\text{weight of product (g)}}{\text{ingredient volume (ml)}} \times 100\%
\] (1)
2.4. Variable observed

The research method used a completely randomized design (CRD) with a factorial experiment. Increase the validity of the final results; each treatment has three replications’ combinations. Data analysis used a mathematical model of the factorial design [10]. If the interaction of factors A and B has an effect, then further tests are carried out using Duncan.

3. Result and discussion

3.1. Result

Different treatments in making nata from palm sugar resulted in different average yield values. The yield value ranged from 28.23% to 42.07%, with an average of 34.31%. The average yield value of nata de arenga production in each treatment combination can see in Table 2.

| Treatment | Repetition | Total | Mean |
|-----------|------------|-------|------|
|           | I          | II    | III  |      |
| F₁Va      | 31.87      | 28.23 | 30.39| 90.49| 30.16|
| F₁Vb      | 33.16      | 32.09 | 33.66| 98.91| 32.97|
| F₁Vc      | 29.70      | 32.61 | 32.59| 94.90| 31.63|
| F₂Va      | 33.84      | 39.16 | 37.89| 110.89| 36.96|
| F₂Vb      | 30.47      | 33.1  | 30.99| 94.56| 31.52|
| F₂Vc      | 35.56      | 35.73 | 34.01| 105.30| 35.10|
| F₃Va      | 31.54      | 36.64 | 31.06| 99.24| 33.08|
| F₃Vb      | 41.77      | 33.51 | 38.96| 114.24| 38.08|
| F₃Vc      | 42.07      | 35.41 | 40.33| 117.81| 39.27|

Figure 1 shows that using a starter with a fermentation time of 8 days as much as 300 ml (F₁Vc) in the making nata de arenga resulted in the highest average yield (39.27%). Meanwhile, using a starter with a fermentation time of 4 days as much as 200 ml (F₁Va) resulted in the lowest average yield (30.16%).

The variance analysis showed that the starter's fermentation time significantly affected the yield produced. However, the volume of starter used has no significant effect on the yield produced. Thus, the interaction between the two treatments gave an authentic effect. Therefore, Duncan's further test does carry out to determine the effect of fermentation time on the yield of nata produced. The results of this test do present in Tables 3 to 8.
Duncan’s test results presented in Table 3 show that the yield produced in the making of nata, using a starter that was fermented for four days as much as 100 ml (F1Va) was not significantly different from the use of a starter of 200 ml (F1Vb) and 300 ml (F1Vc).

**Table 3.** Duncan test results in effect of using a starter fermented for four days with different volumes (100 ml, 200 ml, and 300 ml) on the yield of nata produced.

| Treatment | Average Yield (g) | Duncan's Test |
|-----------|-------------------|---------------|
| F1Va      | 30.6              | a             |
| F1Vb      | 32.97             | a             |
| F1Vc      | 31.63             | a             |

Note: the numbers followed by the same letter are not significantly different in effect.

**Table 4.** Duncan test results in effect of using a starter fermented for six days with different volumes (100 ml, 200 ml, and 300 ml) on the yield of nata produced.

| Treatment | Average Yield (g) | Duncan's Test |
|-----------|-------------------|---------------|
| F2Va      | 36.96             | a             |
| F2Vb      | 31.52             | b             |
| F2Vc      | 3.1               | ab            |

Note: the numbers followed by the same letter are not significantly different in effect.

**Table 5.** Duncan test results in effect of using a starter fermented for eight days with different volumes (100 ml, 200 ml, and 300 ml) on the yield of nata produced.

| Treatment | Average Yield (g) | Duncan's Test |
|-----------|-------------------|---------------|
| F3Va      | 33.08             | c             |
| F3Vb      | 38.08             | ac            |
| F3Vc      | 39.27             | a             |

Note: the numbers followed by the same letter are not significantly different in effect.
Table 4 shows that in the making of nata, the yield of nata produced using a 6-day fermentation starter as much as 100 ml (F_2Va) was not significantly different from the addition of 300 ml starter (F_2Vc) but significantly different from the addition of 200 ml starter (F_2Vb). Meanwhile, using a 6-day fermentation starter as much as 200 ml (F_2Vb) was not significantly different from the addition of 300 ml starter (F_2Vc). Table 5 shows that the yield produced in the making of nata using an 8-day fermentation starter as much as 100 ml (F_3Va) was significantly different with the addition of 200 ml (F_3Vb) and 300 ml (F_3Vc) starter. However, using an 8-day fermentation starter as much as 200 ml (F_3Vb) was not significantly different from adding of 300 ml starter (F_3Vc).

The results of Duncan's test on the effect of differences in fermentation time on adding the same starter volume present in Tables 6, 7, and 8. The results of Duncan's test in Table 6 show that in making nata, the use of starter fermented for four days as much as 100 ml (F_1Va) was significantly different from the use of starter fermented for six days as much as 100 ml (F_2Va) but not significantly different from the use of starter fermented for eight days as much as 100 ml (F_3Va). Meanwhile, a starter fermented 100 ml (F_2Va) for six days was not significantly different from the starter fermented 100 ml (F_3Va) for eight days.

Table 6. Duncan test results in effect fermentation time (4, 6, and 8 Day) starter treatment with the addition of 100 ml starter on the yield of nata produced.

| Treatment | Average Yield (g) | Duncan's Test |
|-----------|-------------------|---------------|
| F_1Va     | 30.16             | b             |
| F_2Va     | 36.96             | a             |
| F_3Va     | 33.08             | ab            |

Note: the numbers followed by the same letter are not significantly different in effect.

Table 7. Duncan test results in effect fermentation time (4, 6, and 8 Day) starter treatment with the addition of 200 ml starter on the yield of nata produced.

| Treatment | Average Yield (g) | Duncan's Test |
|-----------|-------------------|---------------|
| F_1Vb     | 32.97             | b             |
| F_2Vb     | 31.52             | b             |
| F_3Vb     | 38.08             | a             |

Note: the numbers followed by the same letter are not significantly different in effect.

Table 8. Duncan test results in effect fermentation time (4, 6, and 8 Day) starter treatment with the addition of 300 ml starter on the yield of nata produced.

| Treatment | Average Yield (g) | Duncan's Test |
|-----------|-------------------|---------------|
| F_1Vc     | 31.63             | b             |
| F_2Vc     | 35.1              | ab            |
| F_3Vc     | 39.27             | a             |

Note: the numbers followed by the same letter are not significantly different in effect.

In Table 7, Duncan's test shows that in the making of nata, the yield of nata produced using a 4-day (F_1Vb) or a 6-day (F_2Vb) fermentation starter as much as 200 ml was significantly different from the use of a starter that was fermented for eight days as much as 200 ml (F_3Vb). Duncan's test results in Table 8 show that the yield of nata produced at the making nata using a starter that was fermented for four days as much as 300 ml (F_1Vc) was not significantly different from making nata using a starter that was fermented for six days (F_2Vc) but significantly different by making nata using a starter that fermented for eight days (F_3Vc). Meanwhile, making nata using a starter fermented for six days as much as 300 ml (F_2Vc) was not significantly different from making nata using a starter that fermented for 8 (F_3Vc).
3.2. Discussion

Nira of palm sugar contains water content ranging from 80-85% and sucrose around 15%. These conditions are very suitable for the growth of microorganisms. Microorganisms found in the nira are yeast and bacteria. One of the microorganisms present in nira is acetobacter [11].

In this study, the fermentation time affects the yield of nata produced. The highest average yield obtains in the F3Vc treatment. In this treatment, using a starter was fermented for eight days. At the 8-day-old starter, A. xylinum was in the exponential growth phase. This phase, nata bacteria secrete as much extracellular polymerase enzyme as possible to arrange glucose polymers into cellulose (nata matrix) so that in this phase, the maximum nata will form [12].

Another factor that can increase the yield of nata is the volume or concentration of the starter added when making nata. The concentration of starter affects the thickness and yield of cellulose in the making of Nata de Soya [13]. However, in this study, the concentration (volume) of the starter added to the fermentation medium (nira's solution) did not affect the yield produced. Although in this study, the most significant yield was in the treatment with the addition of 300 ml of starter (F3Vc).

The nitrogen source that does generally use in the production of nata comes from Za, an inorganic source. However, the use of Za as a nitrogen source is quite dangerous for consumption. This material is a chemical that functions as a plant fertilizer, not a food consumption ingredient. Therefore, sources of organic nitrogen substitute for Za need to develop to minimize the use of Za in the making of nata. One source of nitrogen from non-Za materials that can use is bean sprouts. This study used 25 g of bean sprouts as a nitrogen source and yielded 39.27%. Using the same raw material (nira of palm sugar), Lempang (2017) using Za as a nitrogen source in making nata, the yield reached 94.22%. Different nitrogen sources in Za and bean sprouts are one factor in the high yield of nata produced [14]. Putranto and Taofik's (2017) research used coconut water to make nata and varying concentrations of bean sprout extract, resulting in the highest yield of 89.97% [15]. The use of various ingredients and the concentration of bean sprout extract is one factor in the high yield of nata produced.

In the above explanation, separately, the age of the starter affects the yield, but not for the volume of the starter added at the time of making nata. However, the interaction between starter age and the volume of starter added to the making of nata affects the yield produced. Using older starter fermentation and the more starter volume added to the making of nata, the higher the yield produced. This condition is an optimal condition where the fermentation time and the number of starters are the primary triggers for the formation of nata. When inoculated into the fermentation medium (nira's solution), the microbial population is large. In addition, the microbes in the fermentation media are in optimum physiological conditions for the nata fermentation process. The increasing the starter's age can indicate that the microbes have multiplied to increase the population [12]. In addition, metabolic activity and cell enlargement will continue in producing nata. The high volume of starter significantly influences the physiological activities of microbes in producing extracellular enzymes that can arrange glucose compounds in the nira solution media into thousands of fiber or cellulose chains (nata) [13]. It indicates that the more volume used, the more bacteria will be active in cell division and cell multiplication so that the cell size will increase.

4. Conclusion

The highest average yield (39.27%) founds in the making of nata using a starter that was fermented for eight days as much as 300 ml (F3Vc). The interaction between starter fermentation time and starter volume affects the yield of nata produced. The longer starter fermentation time results in a higher microbial population; the more starter volume used, the larger the cell size formed. The F3Vc treatment resulted in the highest yield of nata de arenga compared to other combination treatments.

References
[1] Republik Indonesia M K 2007 Peraturan Menteri Kehutanan Nomor: P.35/Menhut-II/2007 Tentang Hasil Hutan Bukan Kayu (Jakarta)
[2] Santoso E, Purwito D, Pratiwi, Pari G, Turjaman M, Leksono B, Widyatmoko A, Irianto R S B,
Subiakto A, Kartonowaluyo T, Rahman, Tampubolon A and Siran S A 2012 *Master Plan Penelitian dan Pengembangan Gaharu Tahun 2013-2023* (Jakarta)

[3] Fatah A and Sutejo H 2015 Tinjauan Keragaan Tanaman Aren (*Arenga pinnata* Merr) di Kabupaten Kutai Barat *J. Agrifor XIV*

[4] Tenda E T, Maskromo I and Heliyanto B 2010 Ekspolrasi Plasma Nutfah Aren (*Arenga pinnata* Merr) di Kutai Timur, Provinsi Kalimantan Timur *Bul. Palma Juni*

[5] Siti Nurhayati 2006 Kajian pengaruh kadar gula dan lama fermentasi terhadap kualitas nata de soya *J. Mat. Sains dan Teknol. 7* 40–7

[6] Mayasti N K I and Ari D 2013 Pemanfaatan Ampas Basah Tapioka Sebagai Media Fermentasi dalam Pembuatan Nata De Cassava *PANGAN 22* 365–72

[7] Harlis, Murni P and Muswita 2015 Pemanfaatan Acetobacter xylinum terhadap Peningkatan Kualitas Nata de Banana Skin *Biospecies 8* 29–33

[8] Yugatama A, Maharani L, Pratiwi H and Ikaditya L 2015 Uji Karakteristik Mikrokrystalin Selulosa dari Nata De Soya Sebagai Eksipien Tablet *Farmasains 2* 269–74

[9] Mayang Sari Y and dan Ketut Budaraga Universitas Ekasakti A I 2017 Pengaruh konsentrasi starter acetobacter xylinum terhadap mutu nata de cucumber *J. Pertan. UMSB 25* 27–3663

[10] Gaspersz V 1991 *Metode Perancangan Percobaan* (Bandung: CV ARMICO)

[11] Barlina R and Lay A 1994 Pengolahan Nira Kelapa untuk Produk Fermentasi Nata de coco, Alkohol dan Asam Cuka. *J. Penelit. Kelapa 7* 21–3

[12] Pambayun R 2002 *Teknologi Pengolahan Nata De Coco* (Yogyakarta: Kanisius)

[13] Budiarti R S 2008 Pengaruh konsentrasi starter acetobacter xylinum terhadap ketebalan dan rendemen selulosa *Biospecies 1* 19–24

[14] Lempang M 2017 Produksi nata pinnata dari nira aren *Info Tek. EBONI 14* 23–33

[15] Putranto K and Taofik A 2017 Penambahan Ekstrak Toge Pada Media Nata De Coco *Agroteknologi X* 138–49