Addition of tape yeast and tempe mould in optimization of traditional coconut oils production

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Abstract. Plik u is fermented, grated, and dried coconut meat which is used as a cooking ingredient in Aceh, Indonesia. This ingredient is a by-product of the manufacturing process of traditional coconut oils which are known as simplah and pliek u oils. These three products are produced by natural fermentation (without the addition of starter cultures) and the whole process takes up to 20 days. Therefore, this study aims to test methods for speeding up production time and for increasing the yield by adding commercial starter cultures (tape yeast, tempe mould, and combination of both types). The coconut was fermented for one to three days. A control was conducted for fermented coconut without adding any starters. This experiment was conducted using group randomised design with three replicates. The results show that the addition of commercial starters decreased the production time up to nine days. The application of a single starter shortened fermentation time to two days while the combination of the starters required three days. In terms of the yield, the use of tape yeast either on its own or in combination starters increased the yield of simplah oil more than 100%. The addition of tempe mould either on its own or in combination starters increased the yield of pliek u oil up to 60%. Conversely, the yield of pliek u was maximised without adding any starters. These findings suggested that tape yeast and tempe mould should not be added at the same time to maximize the production of both oils.

1. Introduction
Coconut is one of main commodities in Indonesia and in Aceh Province, Aceh Besar is the third largest producer with plantation areas as wide as 35,659 acres [1]. Typically, a young coconut is served as a beverage while mature ones are processed into cooking oil. In Aceh, the mature coconut is fermented traditionally into simplah oil, pliek u oil and pliek u dregs. These three products are obtained in one production line consisting of three steps; coconut meat fermentation, sun drying, and pressing. Simpilah oil is produced after fermentation, while pliek u oil and pliek u dregs are produced at the end of the process. Based on a preliminary survey in Pidie District [2], pliek u dregs are made using various methods. Coconuts can be treated by either grating before fermentation or fermenting before grating. The raw
coconut is mature, half mature, or mixed. Then the production time varies from 9 to 18 days with 4-6 rounds of pressing. According to [3], the total time needed to produce simplah oil is around 9 days while pliek u oil and the dregs take about 15-20 days to produce.

In order to optimise pliek u oil production, a method to speed up the process and to increase the yield is required. Researchers have reported that the addition of starter cultures shortens the fermentation process time in other fermented products [4,5]. In virgin coconut oil (VCO), the addition of 20% tape yeast (Saccharomyces cerevisiae) in 4 litres of coconut milk increased the yield from 18% to 24.23% in 10 hours [6]. The combination of the papain enzyme and tempe mould (Rhizopus oligosporus) could also increase yield as much as 27.8% in 24 hours [7]. Furthermore, 35.49% higher yield is obtained in 24 hours with the addition of tape yeast as a starter culture in coconut milk fermentation [8]. [9] used tape yeast and papaya fruit sap for coconut oil extraction and the process took around 18 days. This is because yeast and mould in traditional starter cultures produce lipase enzyme which is important to break down proteins covering fat granules in the oil emulsion.

A long fermentation and drying process should be shortened to reduce the total amount of free fatty acids which cause rancidity in the oil. Simplah oil, pliek u oil, and pliek u dregs are also produced by natural fermentation (without the addition of starter cultures). Therefore, in this study, commercial starter cultures (tape yeast and tempe mould) obtained from a local market were used to optimise the fermentation process and to increase the production of pliek u oil and the dregs. Both starters are obtainable and reasonable in price so they are applicable for pliek u producers. The starters, both on their own and in combination, were added to grated coconut that had been fermented for three days.

2. Materials and methods

2.1. Materials
Fresh and mature coconut of the Dalam variety, tape yeast, and tempe mould were purchased in Lambaro market, Aceh Besar District, Aceh Province, Indonesia.

2.2. Starter culture preparation
Starter culture was prepared by diluting 1 g of grounded tape yeast in 9 ml of buffer pepton water (BPW), vortexing the mixture for 1 minute, and diluting up to $10^{-10}$. A hundred µl of the suspension was spread on Potato Dextrose Agar (PDA) and incubated at 25°C for 48 hours. After incubation, the number of colonies forming units (cfu) on the agar plate was calculated. The calculation was used to determine the amount of starter ($10^{12}$ cfu/g) which would be applied in the production of simplah oil, pliek u oil, and pliek u. The same preparation was applied to tempe mould and the combination starters.

2.3. Production of simplah oil, pliek u oil, and pliek u
This experiment was conducted using group randomised design with two factors and three replicates. The first factor was types of starter culture (tape yeast, tempe mould, combination, and control). The second factor was fermentation time (1 day, 2 days, and 3 days) so there were 36 samples. The production of these three products was conducted at a pliek u home industry in Cot Meulangen Village, Blang Bintang Sub-district, Great Aceh District, Aceh Province, Indonesia.

Two-step fermentation was required to produce simplah oil, pliek u oil, and pliek u. The initial fermentation was performed by placing open coconuts in a gunny sack for 3 days prior to being grated and placed in a plastic bowl. Based on the calculation of cfu, tape yeast (11.11 g), tempe mould (8.96 g), and the combination of the starters (9.75 g) were sprinkled separately on the grated coconut (1 kg). The control was conducted for fermented coconut without adding any starters. The coconut was further fermented for 1 day, 2 days, and 3 days at 25°C [10]. During the fermentation, the fermented coconut was squeezed and positioned alongside the container to let the simplah oil gather in the centre. After fermentation, simplah oil was collected and kept at 27 degrees C in a plastic bottle prior to be analysed. The fermented coconut was sun-dried and pressed until no pliek u oil was produced. The pliek u oil was
kept at the same condition as simplah oil, while the pliek u was kept in a plastic bag. All samples were then analysed for yield.

2.4. Statistical analysis
The statistical analyses were performed using One-way Analysis of Variance (ANOVA). Means that were significantly different at 5% level of probability (P<0.05) were further analysed by Least Significant Different (LSD) on IBM SPSS Statistics 22.

3. Results and discussions
3.1. Optimization of production time
Production time of simplah oil, pliek u oil and pliek u dregs with different treatments can be seen in Table 1. The addition of starter cultures in this study, both on their own and in combination, did not affect the production time of pliek u oil and the dregs (9 days) compared to the control. Further fermentation time affected the drying time. Interestingly, in control, oil could be extracted after 3 days of drying even the coconut was further fermented for one day only. This suggests that the condition of fermentation was suitable for initial microbes to grow without competing with the starter cultures added. Moreover, one day further fermentation prolonged the one day drying time indicating that the production of simplah oil was at a low level in the beginning. The production time of simplah oil is not related to the drying time as it is produced before drying step.

Table 1. Production time of simplah oil, pliek u oil and pliek u dregs with different treatments

| Type of Starter Cultures (R) | Initial Fermentation Time (Days) | Further Fermentation Time (Days; H) | Drying Time (Days) | Production Time (Days) |
|-----------------------------|----------------------------------|-------------------------------------|-------------------|-----------------------|
| Tape Yeast (R1)             | 3                                | 1 (H1)                              | 2                 | 6                     |
|                             | 3                                | 2 (H2)                              | 2                 | 7                     |
|                             | 3                                | 3 (H3)                              | 3                 | 9                     |
| Tempe Mould (R2)            | 3                                | 1 (H1)                              | 2                 | 6                     |
|                             | 3                                | 2 (H2)                              | 2                 | 7                     |
|                             | 3                                | 3 (H3)                              | 3                 | 9                     |
| Tape Yeast + Tempe Mould (R3)| 3                                | 1 (H1)                              | 2                 | 6                     |
|                             | 3                                | 2 (H2)                              | 2                 | 7                     |
|                             | 3                                | 3 (H3)                              | 3                 | 9                     |
| Control (Without Starter Cultures; R4) | 3                                | 1 (H1)                              | 3                 | 7                     |
|                             | 3                                | 2 (H2)                              | 3                 | 8                     |
|                             | 3                                | 3 (H3)                              | 3                 | 9                     |

*aaccumulation of initial fermentation time, further fermentation time, and drying time.
*baccumulation of initial fermentation time and further fermentation time.

However, these figures are lower than that of the traditional fermentation which needs up to 18 days [2,9] or 20 days [3]. Reduction in manufacturing time is desired to maintain oil quality as both oils have a high level of free fatty acids, peroxide value, and moisture content [9]. According to [11], the high peroxide value produced in the oil is due to the natural fermentation process that grows fat-decomposing and fatty acid oxidizing microorganisms. Peroxide value is also increased in pliek u oil because of the oxidation process during drying.
3.2. Optimization of yield

The effect of starter culture type and fermentation time on simplah oil, pliek u oil, and pliek u dregs yield can be seen in Figure 1, 2, and 3 respectively. Figure 1 indicates that the addition of starter cultures, both singly and in combination, increased the yield of simplah oil significantly compared to the control. Without the addition of a starter culture, the highest yield of simplah oil is 2.6%. The percentage is doubled with the addition of the starter. The highest simplah oil yield is obtained by adding tape yeast and the combination of tape yeast and tempe mould. Tape yeast as single starter shows the best performance with 2 days further fermentation. While the combination of the starters reaches the peak with 3 days further fermentation. The addition of tempe mould for 1 day further fermented coconut produces the lowest yield among treatments.

According to [12], S. cerevisiae grows aerobically and anaerobically and has ability to use different sugars depending on which way it grows so tape yeast grows during initial fermentation when the grated coconut is placed in a gunny sack and further ferments when it is placed in a plastic bowl. [13] also mentions that the optimum temperatures for the most rapid initial growth of S. cerevisiae are in the range 30-35 degrees Celsius. The growth of this yeast causes acid production which decreases pH. Based on [8], at a low pH level (4-5), protein clumping affects the separation between protein and oil. Tape yeast also produces a lipase enzyme which destroys fat globules. Therefore, simplah oil is produced largely because of tape yeast addition. This finding is different than a study conducted by [9] where the yeast added might not reach 10^12 cfu/g when it was added.

As for pliek u oil, the best starter to increase yield is tempe mould and its combination with tape yeast. Figure 2 shows that the addition of tempe mould, both singly and in combination, raises the yield of pliek u oil considerably compared to the control. Interestingly, the performance of tempe mould declines gradually as the fermentation time gets longer.

The most rapid growth of Rhizopus oligosporus (tempe mould) occurs at pH values less than 6 [14], temperatures at about 40°C and O2 concentration above 1% [15]. According to [16], the growth of this mould is not significantly influenced by any lactic acid bacteria (Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus reuteri and Lactococcus lactis) when these bacteria are present during fermentation. Therefore, the finding of this study recommends that tempe mould should not be added at the beginning of the process. Tempe mould can be added in the further fermentation or before the drying and pressing steps. In these steps, the temperature and pH of the early stage fermented coconut
were higher than that of the grated coconut, but were lower than that of the final fermented coconut (unpublished data). In contrast, the addition of starter cultures, both singly and in combination, decreased the yield of pliek u dregs (Figure 3). The control produced 23.8% yield while the treated coconut only produced 20% yield. This result supports that production of both oils was optimized because of the addition of starter. However, further research on the quality of these three products is necessary.

Figure 2. Effect of starter culture type (R) and fermentation time (H) on pliek u oil yield (notation with the same letter shows no difference)

Figure 3. Effect of starter culture type (R) and fermentation time (H) on pliek u dregs yield (notation with the same letter shows no difference)

4. Conclusions
Overall, the addition of commercial starters in fermented, grated, and dried coconut shortened the production time. Moreover, different commercial starter cultures were needed to increase the yield of fermented coconut meat products from Aceh. While tape yeast is necessary to increase simpelah oil yield and should be added before further fermentation, tempe mould is necessary to increase pliek u oil yield and should be added in the further fermentation process or before the drying and pressing steps. Also, the yield of pliek u dregs was maximised without the addition of both starter cultures.
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