Palm oil stripping through cellulolytic microorganism fermentation

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Abstract. Fresh fruit bunch (FFB) is sterilised to inactivate the enzyme, facilitate the release of fruit from fruit bunch, and soften the fruit for ease of oil release from mesocarp. Nevertheless, sterilisation heat can damage the oil. In this study, a modification of the oil palm processing was done to facilitate the release of fruit from FFB by using cellulolytic microorganisms through the fermentation process. This study aims to obtain the optimum inoculum and fermentation time in the processing of palm fruits to produce palm oil with high quantity and quality. The types of inoculum used were Bacillus subtilis, Aspergillus niger, Trichoderma harzianum, and a combination of B.subtilis+A.niger, and B.subtilis+T.harzianum. Fermentation was carried out for 20, 40, and 60 hours. The fruit that has been separated from the fruit bunch was then extracted using n-hexane. The results showed that the interaction between inoculum types and fermentation time had a significant effect on oil yield, free fatty acid content, deterioration of bleaching index (DOBI), beta carotene content, and peroxide value. The effective treatment to produces an oil with optimum quantity and quality was using T.harzianum, fermented for 20 hours.

1. Introduction

There are several processing steps of the fresh fruit bunches (FFB) before going to the palm oil mill. These steps include harvesting and transporting of FFB from field to factory for processing. In the palm oil processing, stripping was carried out by the sterilisation process to soften the fruits, and the fruitlets were separated from bunches. These activities need careful attention to avoid increasing free fatty acid (FFA) content in the FFB [1]. The oil quality is indicated by a low FFA content and low peroxide value, high deterioration of bleachability index (DOBI), and the lower moisture and impurities content [2]. Therefore, the processing of palm oil should be done at a lower temperature and the possible shortest times [3].

Improper handling of FFB, both in the field and at the mill, reduces the quality of crude palm oil (CPO) [4], resulting in contamination that causes hydrolysis and oxidation. The higher the temperature used, in the processing, the faster the damage reaction will occur. The oxidation rate process is doubled for every 15 °C rise in the temperature [3]. During the palm oil processing, quality degradation occurs throughout the process, such as in steriliser, screw press, digester, and clarifier. The sterilisation process affects oil quality, especially FFA and bleachability [5]. During sterilisation,
FFB is subjected to high temperature for a long time which causes rapid oxidation. Research by [6] showed that the presence of oxygen during sterilisation led to increased FFA and decreased bleachability. Therefore, fermentation at low temperature may be beneficial compared to sterilisation. The objective of this study was to evaluate the effect of fermentation process with various cellulolytic microorganisms.

2. Materials and Methods

2.1. Sample preparation
The ripe oil palm FFB from Tenera species were obtained from Sekolah Tinggi Ilmu Pertanian Agrobisnis Perkebunan Medan. The weight of each FFB was around 10-12 kg.

2.2. Inoculum preparation
Preparation of potato dextrose broth (PDB) are as follows: 7.2 g of PDB were dissolved in 300 ml of distilled water and was autoclaved at 121°C for 20 minutes. A. niger, T.harzianum, and B.subtilis were inoculated and incubated at 30°C for seven days at 80 rpm in an orbital shaker. The growth of A. niger, T.harzianum, and B.subtilis was analysed by measuring optical density at 580nm in a spectrophotometer.

2.3. Fermentation of oil palm bunches
Oil palm bunches were inoculated with A.niger, T. Harzianum, B.subtilis, mix culture of B.subtilis + A.niger and B.subtilis + T.harzianum for 20, 40, and 60 hours in a fermentor (wooden box) at room temperature. After each fermentation time, the fruit-lets were collected while the non-detached fruits were released manually from the bunch.

2.4. Oven drying
The fruitlets were dried in an oven (Memmert, USA) at 103 °C for 24h to remove the moisture before extraction.

2.5. Oil extraction
Palm oil extraction was carried out using MPOB Test p2.5 methods [7]. The dried fruitlets were cut, sliced and the nut was removed to get the mesocarp. 20g mesocarp of oil palm fruits were put into a Soxhlet Extractor Thimble, and 300ml hexane was used as a solvent. The extraction process was done at 60 °C for 4h until the yellow colour of oil disappears. The solvent was evaporated at 60°C, and oil was dried at 103 °C for 2h to remove any moisture. Crude oil samples from each treatment were weight to get the oil yield and then determined the FFA, beta carotene, DOBI (deterioration of bleachability index), and peroxide value.

2.6. Free fatty acid (FFA) content
Oil samples were determined for their FFA content using acid-base titration by MPOB Test Methods p2.5 [7]. The sample was preheated until its temperature reaches 50 °C. 2,5g preheated oil was put into a volumetric flask and diluted with neutralised ethanol to the 25 mL mark. FFA of oil was neutralised with sodium hydroxide 0.1N. FFA content was expressed as palmitic acid (%); the predominant fatty acid in palm oil.

2.7. Beta carotene content
Beta carotene content was analysed using MPOB test methods p2.6 [7]. 0.04g oil was diluted with n-hexane to the 25ml mark. Solution absorbance was read at 446 nm using UV-vis spectrophotometer (Genesys). The beta carotene content of samples was expressed as ppm using the following equation:

\[ \beta - \text{carotene (ppm)} = 25 \times \frac{383}{100W} (a_s - a_b) \]
where $a_s$ is sample absorbance, $a_b$ is cuvette error, and $w$ is sample weight in gram.

2.8. Deterioration of bleaching index (DOBI)

DOBI was analysed by using MPOB test methods p2.9 [7]. DOBI is the ratio of the absorbance of oil at 446nm to that 269nm. 0.1g oil sample was diluted with n-hexane to the 25ml mark. The absorbance of the sample was read at 446nm and 269nm using UV-vis spectrophotometer (Genesys), and a mean value from duplicate reading was calculated.

2.9. Peroxide value

The determination of peroxide value was carried out by using AOCS methods, Cd 8-53 [8]. 5g sample was put into a 250mL Erlenmeyer flask with a glass stopper, and 30mL 3:2 acetic acid-chloroform solution was added and stirred to dissolve the sample. Saturated KI solution (0.5 mL) was added using a volumetric pipet. The solution was left for 1 min with occasional stirring, and then immediately 30 ml of distilled water was added before the sample was put in the titrator. The solution was titrated with 0.1N sodium thiosulfate by using 1mL starch solution as an indicator. The titration was stopped until the yellow colour disappears. The blank titration was made and must not exceed 0.1mL of the 0.1N sodium thiosulfate solution. The peroxide value of oil was expressed in meq/kg and calculated by using the following equation:

$$\text{Peroxide value} = \frac{(S-B) \times N \times 1000}{w}$$

(2)

where: B is titrant volume of blank (ml), $S =$ titrant volume of sample (ml), $N= $ normality of sodium thiosulfate solution.

2.10. Statistics

Data analysis was performed by Analysis of Variance (ANOVA) using the F test. Significance was established at 5% probability for comparing the means between treatments using least significant differences.

3. Results and Discussion

3.1. Oil yield

In this study, the Soxhlet extraction process was carried out following the method of MPOB Test Methods p2.5 [7]. The influence of the types of microorganisms and fermentation duration on the yield of palm oil produced can be seen in Table 1. Table 1 shows that the longer the fermentation process, the yield of oil produced tends to decrease. The use of $B$. $subtilis$ in a single inoculum resulted in lower oil yields compared to using a mixture of $B$. $subtilis$ with $A$. $niger$ or $T$. $harzianum$. However, the use of $T$. $harzianum$ in the form of a single inoculum actually resulted in the highest oil yield at 20 hours of fermentation time. $B$. $subtilis$, $A$. $niger$ and $T$. $harzianum$ are cellulolytic microorganisms [9,10,11] that can degrade cellulose in oil pam fruit bunches so that it is easier to detach from the bunches, and the oil becomes easier to extract.

3.2. Free fatty acid (FFA content)

The effect of inoculum type and fermentation time on the FFA content of oil extracted is shown in Figure 1. The FFA content of extracted oil in this research were 0.61-2.29%. The longer the fermentation increased the FFA content. This happens due to the activity of the lipase enzyme that appears breaks down fat into fatty acids—the lowest FFA content found in a $L$. $subtilis$ fermentation for 20 hr. According to the CPO quality standard [12], the FFA content should be no more than 5%.
Table 1. Effect of inoculum type and fermentation time on oil yield.

| Inoculum Type                  | Fermentation Time (hours) | Palm Oil Yield (%) |
|--------------------------------|---------------------------|--------------------|
| *Bacillus subtilis* (BS)       | 20                        | 77.78±1.74d        |
|                                | 40                        | 70.38±0.78e        |
|                                | 60                        | 65.23±1.38g        |
| *Aspergillus niger* (AN)       | 20                        | 82.10±1.71c        |
|                                | 40                        | 76.61±1.86d        |
|                                | 60                        | 67.60±1.75f        |
| *Trichoderma harzianum* (TH)   | 20                        | 88.18±1.52a        |
|                                | 40                        | 81.48±0.53c        |
|                                | 60                        | 67.93±0.81f        |
| BS+ AN                         | 20                        | 86.64±1.39b        |
|                                | 40                        | 78.21±1.29d        |
|                                | 60                        | 58.19±1.35i        |
| BS+ TH                         | 20                        | 85.49±1.47b        |
|                                | 40                        | 67.74±1.53f        |
|                                | 60                        | 61.63±0.99h        |

Values in the table are averages of 3 replicates, ±standard deviation. Different letter notation in the same column shows a significantly different effect at 5% level.

3.3. Beta carotene content

Figure 2 shows the Beta-carotene extracted from palm oil. The longer the fermentation, the lower the beta carotene concentration. Different types of microorganisms separated oil with different beta-carotene content. The levels of beta-carotene produced ranged from 127.38 – 373.93 ppm, lower than the original beta carotene value which is estimated at 500-800 ppm [12]. The highest levels of beta-carotene were obtained by fermentation with *Trichoderma harzianum* for 20 hours. The excessive thermal processing may cause the lower beta carotene content in this study during oil extraction [13]. The beta carotene content of oil contributes to the stability and nutritional properties of palm oil. Similar results of the loss of carotene content also reported by Kasmin et al. [14].
Figure 2. Effect of microorganism type and fermentation time on beta carotene content of palm oil (Error bar = ±standard deviation, different letter notation shows the significantly different effect at 5% level).

3.4. Deterioration of Bleaching Index (DOBI)

The DOBI is the ratio of the carotene content to the content of secondary oxidation products. In this study, the DOBI ranged from 0.420 – 1.458, and these values are under the standard quality of CPO. CPO, with a DOBI number between 1.78-2.30, has low quality and challenging to be refined [16].

Figure 3. Effect of microorganism type and fermentation time on the deterioration of bleaching index (DOBI) of palm oil (Error bar = ±standard deviation, different letter notation shows the significantly different effect at 5% level).

Figure 3 showed that the highest DOBI value was obtained in oil from T. harzianum fermentation for 20 h. This result is in line with the beta carotene content. Low DOBI is indicated by the pale or faded orange colour of CPO. The pale colour is due to the decreased carotene content in CPO. The high content of beta-carotene in CPO reflected the high DOBI value. The higher initial content of beta carotene will benefit from decreasing the oxidation rate. However, when the oil loses its beta carotene content, and the oil colour will become pale caused by the low DOBI [15].

3.5. Peroxide Value

The effect of inoculum type and fermentation time on the peroxide value of extracted oil is shown in Figure 4. The highest peroxide number was found in T. harzianum fermentation for 60 h, which was 55.77 meq/kg. Trichoderma effectively controls pathogens considering its ability to produce...
peroxidase and polyphenol oxidase enzymes [16]. Peroxide enzymes can oxidise unsaturated fatty acids to form peroxides. Also, the peroxide enzyme can oxidise the saturated fatty acids in the beta carbon bond, thus forming ketone acids and finally methyl ketones. The higher peroxide value was found in the oil from A.niger fermentation due to the capability of this mould to produce a lipoxygenase enzyme [17]. Lipoxygenase is an oxidised enzyme that binds the oxygen with an unsaturated fatty acid such as arachinodin, linoleic, and linolenic acid to produce peroxide and hydroperoxide [18]. The lowest peroxide number was found in the BS+AN treatment for 20h, which was 12.97 meq/kg. The faster the processing of fruit into oil can prevent an increase in water content, reducing free fatty acid binding to oxygen resulting in low peroxide number.

![Figure 4. Effect of microorganism type and fermentation time on peroxide value of palm oil (Error bar = ±standard deviation, different letter notation shows significantly different effect at 5% level).](image)

4. Conclusions
The fermentation of fresh fruit bunches of palm oil by using cellulolytic microorganisms, namely Bacillus subtilis bacteria, mould Aspergillus niger and Trichoderma harzianum can be used to assist palm oil processing. Fermentation using Trichoderma harzianum cultures for 20h presents the highest oil yield with high beta carotene content and DOBI, low FFA and more stable for the oxidative process.

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