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Extraction of Siwalan Oil (*Borassus flabellifer L.*) by Different Methods

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Abstract. Siwalan *(Borassus flabellifer L.)* is one of the major commercial crops in North Coast of Java Island Indonesia, which gives many useful products to the inhabitants. As a plant belonging to palm trees, siwalan fruit has the potential to produce derivative products such as fruit oil, besides being consumed fresh. This study aimed to obtain the most optimal Siwalan fruit oil extraction method using centrifugation, enzymatic, and fermentation methods. Based on the experimental design, the highest yield (1,151%) of the centrifugation method was shown in centrifugation speed 12,000 (rpm) with centrifugation time 60 minutes. The enzymatic method obtained the highest yield (1,021%) with incubation treatment temperature 40°C and concentration of crude papain 3% (b / v), while the highest fermentation method (1,061%) was obtained in 48 hours fermentation time with 3% yeast concentration (b / v). The overall method used showed that the centrifugation method was the most optimal Siwalan oil extraction method.

INTRODUCTION

Vegetable oil is one of the products that was heavily involved in the daily activities of households and industry. In general, the community has known many vegetable oil products such as coconut oil, corn oil, soybean oil, and palm oil as the dominant vegetable oil products found in the market. Based on data acquired from the Indonesian Palm Oil Association (2016), the consumption level of vegetable oils in the world has increased by 15-20% per year, in line with the high global demand. The data show that the effort to develop vegetable oils from new sources is still quite potential.

Siwalan as a palm plant becomes one of the many plants spread in coastal areas with calcareous dry plains. This plant is known to produce fruit that is generally consumed fresh. This plant have the character of a coconut-like fruit measuring 10-18 cm with black outer skin and coir yellowish white with fruit shapes clustered in bunches. Siwalan fruit has two to four fruit seeds coated with thin brown skin. Young siwalan fruit has soft white fleshy fruit seeds with clear liquid in the middle of the seeds. While old siwalan fruit has thick and hard seed flesh resembling coconut meat. With older age of siwalan fruit, it affects the content of oil and vegetable fats found in fruit flesh [15].

Vegetable oil extraction from plant sources of palm species can be carried out in various ways including mechanical methods, enzymatic methods, and the use of microorganisms. Each extraction method produces unique product characteristics in terms of appearance (clarity and color), aroma, taste and fatty acid content of the product [6]. This study aims to develop siwalan vegetable oil and determine the most effective extraction method with the best product characteristics.

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MATERIALS AND METHODS

The conducted research was experimental using a quantitative method approach. Experimental research was carried out to determine the experimental design for each alternative extraction method of fruit oil, thus obtaining the most optimal extraction method.

Materials

The fresh and mature Siwalan fruit were obtained from a local market in Gresik, East Java, Indonesia. The yeast (Saccharomyces cerevisiae) was obtained from a commercial brand, Ragi Tape NKL, bought from a local bakery. Crude papain for enzymatic method was extracted from the armpit of papaya leaf midrib. Other materials used was clean water or distilled water.

The equipment used in this study included stainless steel knives, bowls, ovens, coconut graters, plastic basins, filters, measuring cups, stirrers, measuring flasks, drop pipettes, Erlenmeyer, glass beakers, measuring cups, weighing bottles, analytic balance and exicators.

Methods

Centrifugation Method

In this experiment, Siwalan milk was obtained from the extraction process of fresh and mature fruit and underwent centrifugation process right after extraction. The sample were filtered with filter paper before centrifugation. There were no chemicals used in the extraction process. Rotary wedge cutter was used to emit white siwalan kernel. The size of the filter paper was Grade 104 from Millipore filter paper with 11 μm particle retention. Siwalan milk was filtered through filter paper before it was prepared for the centrifugation process.

Effect of Centrifugation Speed on Production of Siwalan fruit oil

The experiment was started by using filtered and centrifuged siwalan milk. 50ml of siwalan milk was centrifuged according to the parameters set. After centrifuged, the upper layer part of the oil was taken out to measure the yield of oil by percentage. The yield of oil was determined in percentage according to the following Equation 1.

$$A = B / C \times 100\% \qquad (1)$$

From the equation, A is yield percentage of siwalan oil, B is the volume of oil extracted (mL), and C is volume of siwalan milk (mL). The test was repeated by changing the speed of the centrifuge which were 6000, 9000, and 12000 rpm.

Effect of Centrifugation Speed on Production of Siwalan fruit oil

In this experiment, 50 mL fresh siwalan milk was used. PP tube containing fresh siwalan milk was gently placed into the centrifuge. The fresh siwalan milk was centrifuged at different time intervals starting at 15 minutes. This was repeated at 30 min, and 60 min. After centrifugation, the upper layer part of the oil was taken out to measure the yield of oil by percentage. The yield of oil was determined in percentage according to Equation 1.

Enzymatic Method

In this experiment, siwalan fruit oil was produced by fishing method which involves a simple chemical reaction. This method involved heating low temperatures or without using heating. Papain enzyme was used to break siwalan

emulsifying proteins by catalyzing the protein breakdown reaction by hydrolyzing peptide bonds into simpler compounds [9].

Making Siwalan Oil with a Fishing Method

Siwalan grated meat was squeezed using warm water with a ratio of 1: 3 (3 times extraction). Siwalan milk was left for 1-2 hours to separate cream and siwalan milk. Separated siwalan cream was used as an enzymatic ingredient in making siwalan oil.

Enzymatic Extraction Process

Siwalan cream was added with crude papain with variations in addition (% b/v of siwalan milk cream), which were 1%, 2%, 3% and incubation temperature was room temperature (estimate 30°), 40°C, and 50°C. Stirring continued until homogeneous for 20 minutes. Incubation was carried out for 12 hours, thus forming 3 layers. The top layer was separated because it was siwalan oil.

Fermentation Method

In this experiment, fresh siwalan milk was added with distilled water with 1:1 ratio. In every liter was added yeast (*Saccharomyces cerevisiae*) with a variation of 1.0%, 2.0% and 3.0% (% b/v of siwalan milk cream) as an inoculum for the fermentation process. The mixture was made homogeneous by mixing it tightly and left to stand with a time variation of 24, 36, and 48 hours at room temperature. During the stand process, the mixture was separated into several layers with siwalan oil in the top layer. The oil obtained was filtered with zeolite, which functioned to filter while absorbing unpleasant odors and reducing water content.

Calculation of Process Efficiency

Siwalan oil moisture was determined by hot air oven method [3] and oil content with Soxhlet method [2]. The efficiency of the method was calculated using the following Equation 2.

$$Efficiency\ of\ the\ method\ (\%) = \frac{Yield\ Percentage\ by\ dry\ bases}{Oil\ Content\ percentage\ estimated\ by\ soxhelt\ method} \quad (2)$$

Statistical Analysis

All parameters were carried out in triplicate. Statistical mean of three values were presented in the study. Significant differences between means were determined by Duncan's multiple range tests and were significant when $P \leq 0.05$, based on SPSS software.

RESULTS AND DISCUSSION

Effect of Centrifugation Speed on Production of Siwalan Oil

At centrifugation time of 15 minutes according to Table 1 and Fig. 1, it was found that the yield of Siwalan Oil increased gradually when the centrifugation speed increased from 6000 rpm to 12000 rpm. The centrifugation

method managed to produce the Siwalan oil. The oil was able to be separated from fresh Siwalan milk even though the centrifugation time was only 15 minutes. Furthermore, the results showed that the maximum yield of Siwalan oil produced with 15 minutes centrifugation time was 1.151% with a standard deviation of ±0.01015.

Based from Table 1 and Fig. 2, the highest yield was obtained at 12000 rpm at 30 minutes. The higher the centrifugation speed, the higher the yield of Siwalan oil would be. At 30 minutes of centrifugation with 12000 rpm, the Siwalan oil yield increased significantly when the speed of centrifugation was increased. As a result, the yield of Siwalan oil was at maximum value when centrifugation speed of 12000 rpm was used. The mean value of Siwalan oil at centrifugation speed of 12000rpm was 1.113% with a standard deviation of ±0.00954.

The graph of 60 minutes of centrifugation time in Fig. 3 indicated that the Siwalan oil yield increased steadily as the centrifugation speed increased. The Siwalan oil yield escalated sharply when the centrifugation speed increased from 6000 rpm to 12000 rpm. The Siwalan oil yield increased from 1.012% to 11.151%. At this stage, Siwalan oil was produced in high amount due to enough force on breaking the emulsion on the surface of the siwalan milk to produce Siwalan oil. The curve for the yield of Siwalan oil against centrifugation speed at centrifugation time of 15 and 30 minutes were almost similar as both curves showed the highest peak at 12000 rpm. Further increased in the centrifugation speed increased the yield of Siwalan oil. This was explained when the emulsion was formed by homogenizing pure oil and pure water together, these two phases usually rapidly separated into a system that consisted of a layer of oil (lower density) on top of a layer of water (higher density). Thus, the droplets tended to merge with their neighbor when they collided with each other, which eventually led to complete phase separation. The trend in increasing centrifugation speed resulted in the increase of the rate of sedimentation and the emulsion separation of two immiscible liquids [5].

TABLE 1. Yield of Siwalan Oil with Various Extraction Methods

Methods	Variable 1		Variable 2		Design experiment	Oil yield on wet basis (%)	Moisture content of oil (max 0.5%)	Efficiency of Process (%)
Centrifugation Method	Centrifugation speed (rpm) (R)	6000	Centrifugation interval (min) (T)	15	R1T1	0.953^{a}	0.31	99.69
				30	R1T2	0.991^{b}	0.30	99.70
				60	R1T3	1.012^{bc}	0.30	99.70
		9000		15	R2T1	0.985^{b}	0.32	99.68
				30	R2T2	1.037^{c}	0.30	99.70
				60	R2T3	1.065^{c}	0.29	99.71
		12000		15	R3T1	1.048^{c}	0.31	99.69
				30	R3T2	1.113^{d}	0.30	99.70
				60	R3T3	1.151^{e}	0.29	99.71
Enzymatic Method	Incubation temperature (°C) (C)	30	Crude papain addition (% b/v) (P)	1	C1P1	0.963^{a}	0.32	99.68
				2	C1P2	0.992^{b}	0.32	99.68
				3	C1P3	1.018^{bc}	0.33	99.67
		40		1	C2P1	0.981^{b}	0.35	99.65
				2	C2P2	0.988^{b}	0.36	99.64
				3	C2P3	1.021^{bc}	0.36	99.64
		50		1	C3P1	0.989^{b}	0.36	99.64
				2	C3P2	0.997^{b}	0.37	99.63
				3	C3P3	1.013^{bc}	0.37	99.63
Fermentation Method	Fermentation time (hour) (H)	24	Yeast concentration (% b/v) (Y)	1	H1Y1	0.873^{a}	0.41	99.59
				2	H1Y2	0.991^{b}	0.41	99.59
				3	H1Y3	1.016^{bc}	0.42	99.58
		36		1	H2Y1	0.911^{a}	0.44	99.56
				2	H2Y2	1.026^{c}	0.43	99.57
				3	H2Y3	1.055^{c}	0.44	99.56
		48		1	H3Y1	0.982^{b}	0.45	99.55
				2	H3Y2	1.053^{c}	0.46	99.54
				3	H3Y3	1.061^{c}	0.46	99.54

Description: Different letters within same row indicate significant difference between means (P<0.05).

Effect of Centrifugation Times on Production of Siwalan oil

Time was one of important factors that influenced the results of Siwalan oil. Three centrifugation times used were 15, 30, and 60 minutes. Fig. 4 shows the plot of Siwalan oil yield on the time of centrifugation. The highest yield of Siwalan oil at 60 minutes was due to rapid separation of centrifugation speed. The movement from 15 minutes to 60 minutes had an impact on increasing the yield of Siwalan oil. At 15 minutes, Siwalan oil yield was minimal, 0.953% at 6000 rpm. The separation process could be separated from the emulsion. The reason was the longer the separation process, the more oil droplets could be separated from the emulsion.

Proteolytic Activity of Crude Papain for Enzymatic Method

The analysis result of crude papain proteolytic activity was 298.7 MCU/gram (Milk Clotting Unit/gram). The activity of the papain enzyme was relatively high, thus the crude papain enzymes made could be used for Siwalan oil making. Papain, which was produced from the latex of papaya stems and leaves, turned out to have proteolytic activity of about 200 MCU/gram, whereas from the fruit portion was around 400 MCU/gram [9].

Siwalan Oil Yield of Enzymatic Method

Based on the results of variance analysis, there was a real interaction between the addition of coarse papain and the incubation temperature of the resulting Siwalan oil yield. The relationship between coarse papain concentration and incubation temperature in yield is presented in Fig.5

Fig.5 shows that the higher of crude papain addition and the higher of incubation temperature resulted Siwalan oil yield increased. In the addition of 1% crude papain, 0.989% Siwalan oil was obtained with a temperature incubation of 50°C. The use of 2% crude papain (b/v) obtained 0.997% Siwalan oil yield with temperature incubation of 50°C, while the highest yield in the Enzymatic method was 1.021% with the addition of 3% crude papain at a temperature incubation of 40ºC. This showed that the higher the enzyme added, the more peptide bonds in the Siwalan protein that surround the oil could be hydrolyzed, because papain enzyme is a proteolytic enzyme that can hydrolyze peptide bonds [12].

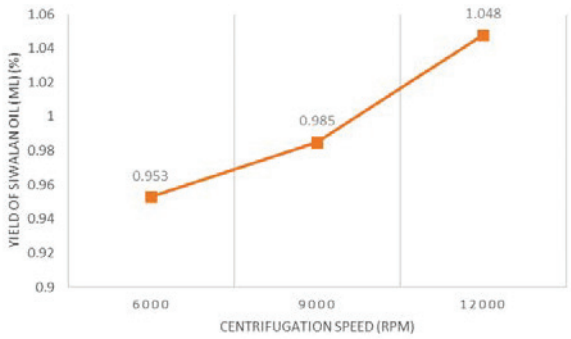

FIGURE 1. Results of Siwalan oil yield (%) under different centrifugation speed (rpm) at 15 minutes

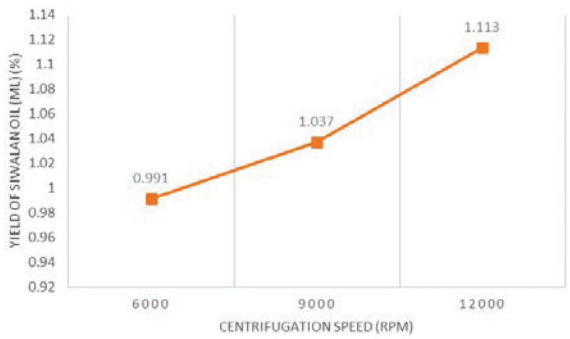

FIGURE 2. Results of Siwalan oil yield (%) under different centrifugation speed (rpm) at 30 minutes

FIGURE 3. Results of Siwalan oil yield (%) under different centrifugation speed (rpm) at 60 minutes

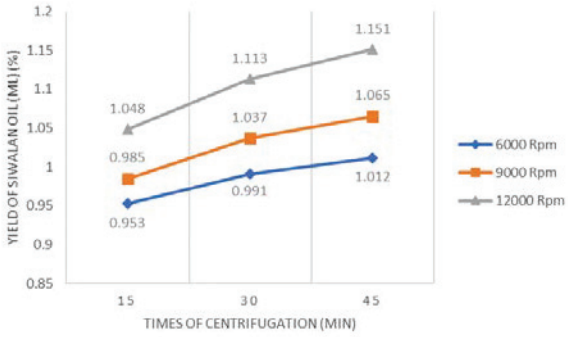

FIGURE 4. Results of Siwalan oil yield (%) under different time of centrifugation (minutes)

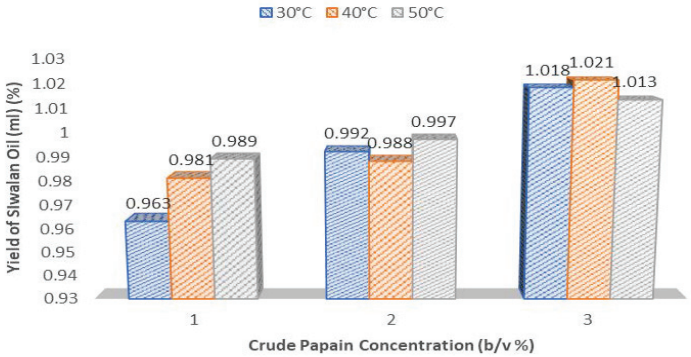

FIGURE 5. Yield of Siwalan oil with Enzymatic Method

The higher the incubation temperature, the faster the speed of the protein hydrolysis reaction, thus the oil that can be released from the protein envelope also increased. The crude papain enzyme was a proteolytic enzyme, which is an enzyme that catalyzes the reaction of breaking the peptide chain in proteins into simpler compounds [16]. Papain enzyme can work optimally at temperatures between 50-60°C and pH between 5-7 [1].

Siwalan Oil Yield of Fermentation Method

In the fermentation method, crude Siwalan oil was formed because of the phenomenon of protein digestion that played a role to stabilize emulsion of the Siwalan cream into a soluble material. Enzymatic starters with high amylolytic and proteolytic capacities could hydrolyze carbohydrates and proteins contained in the Siwalan cream as its substrate into soluble sugar and amino acid and peptide [13]. The extraction process of Siwalan oil via fermentation or enzymatic system involved microbial cell and enzymes those could solve the emulsion. However, their activities were influenced by several factors including substrate, temperature, and incubation period [10].

Preliminary step on extraction process of Siwalan oil was initiated after separating the Siwalan cream, which had higher in lipid content, from Siwalan skims which was higher in carbohydrate and protein content. After addition with starter followed by overnight fermentation of the Siwalan cream at room temperature (30°C), the starter containing enzymes were stimulated to digest starch and ferment it into alcohol and organic acids that coagulate protein in consequence of phases formation of oil on upper part, protein in the middle and water layer on lower part. Due to a lower molecular weight, the oil part formed through the process could be directly separated from protein and water part by draining off both through a valve. To reduce interference of water content or insoluble materials

into the oil part, a further process of obtaining oil by refining through filter paper or vacuum filter and rinse with hot water following by vacuum evaporation was required to avoid chemically processing to achieve the oil [8].

Effect of Inoculum concentration on Production of Siwalan Oil

Inoculum concentration of 1, 2, and 3% were studied to determine its effect on yield. Inoculum concentration of 3% was shown to have more efficient result with 1.004% and poor with the 1% with 0.922%, it showed that the efficiency was directly proportional to the inoculum concentration. Some studies reported that the inoculum concentration of 3% was suitable to produce higher yields [4] [8]. The contented yields (1.023) were reported at 2% inoculum. The concentration of inoculum between 2% and 3% shown that the yields differences were not significant ($P \leq 0.05$) (less than 2%). Therefore, 2 % inoculum concentration was preferable in the Fermentative production of Siwalan oil by Saccharomyces cerevisiae. The values are presented in Table 1 and effect of inoculum concentration shown in the Fig.6.

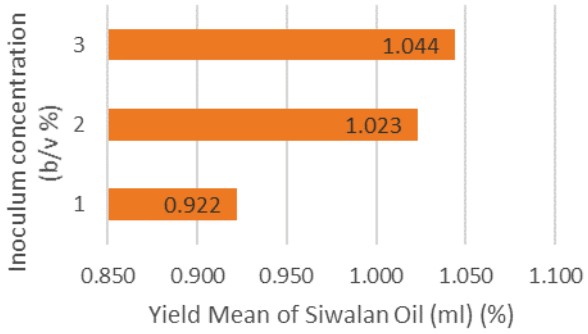

FIGURE 6. Results of Siwalan oil yield (%) under different Inoculum concentration (b/v %)

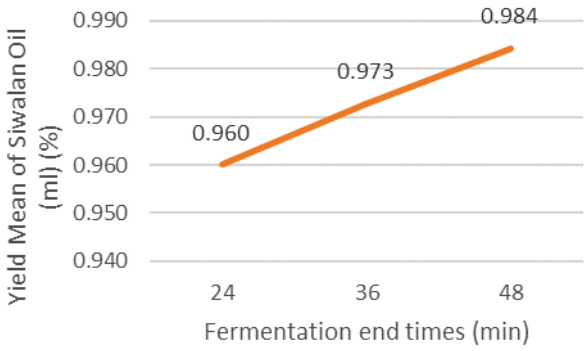

FIGURE 7. Results of Siwalan oil yield (%) under different fermentation end time (minutes)

Effect of Fermentation End Time on Production of Siwalan Oil

The fermentation end times used in this study were 24, 36, and 48 hours. Poor separation was obtained at 24 hours with 0.960% of Siwalan oil yield, but the efficiency for fermentation time 36 h and 72 h slightly increased to 0.973% and 0.984% respectively. The fermentation time of 24 hours increased between 36 and 48 hours, by considering the time factor the optimum fermentation end time could be considered as 48 hours [11]. The increase in

Siwalan oil yield occured with increasing fermentation end time. The results are presented in the Table 1 and the effect of fermentation end time is shown in Fig. 6 and Fig. 7.

CONCLUSION

There were three methods used for Siwalan oil extraction in this study, including centrifugation, enzymatic, and fermentation methods. Based on the experimental design, the highest yield (1,151%) of the centrifugation method was shown in centrifugation speed 12000 (rpm) with centrifugation time 60 minutes. The enzymatic method obtained the highest yield (1,021%) with incubation treatment temperature 40°C and concentration of crude papain 3% (b / v), while the highest fermentation method (1,061%) was obtained in 48 hours fermentation time with 3% yeast concentration (b / v). The overall method used showed that the centrifugation method was the most optimal Siwalan oil extraction method. The yield of Siwalan oil was quite low when compared to other types of palm plants.

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