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Fermentation of Coconut Milk into Virgin Coconut Oil Using Yeast for Biodiesel Production

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Virgin coconut oil (VCO) widely used in food industries, pharmaceuticals as well as beauty industries. Currently, the source of fossil fuels is facing extinction and is predicted to last for only another 50 years. Biodiesel produced by VCO has been indicated to be the perfect substitute for diesel. However, the methods for producing VCO such as heating cost a lot money, energy and time. The fermentation method has been shown to be the most convenient method for producing VCO. This research aims to find the best method for producing VCO for biodiesel production. Specifically, this research aims to determine the yield of VCO produced by the fermentation technique using four types of yeast namely bread yeast, tapai yeast, tempeh yeast and bread yeast with the addition of pineapple extract at different times of fermentation. The result of the organoleptic test, free fatty acids (FFA) test, and iodine test were compared, and VCO was then converted into biodiesel by transesterification using different amounts of catalyst to calculate its percentage yield. The results show that the highest yield percentage of VCO is 28.1% via the fermentation of tapai yeast for 18 hours with an FFA value of 0.8%. For biodiesel production, the transesterification of VCO produced using tapai yeast with 0.3 wt% potassium hydroxide (KOH) showed the highest yield percentage at 7 minutes (81.9%). In conclusion, the fermentation of coconut milk using 0.2 g of tapai yeast at 18 hours is the best method for producing VCO. Additionally, transesterification using 0.3 wt% KOH at 7 minutes is the best condition for biodiesel conversion.

Keywords: Virgin coconut oil; biodiesel; fermentation; transesterification; yeast

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Coconut is a tropical fruit that is used in food and beverage industries worldwide such as for the production of biscuits, drinks, instant coconut milk, and vegetable oil namely VCO [1]. In addition, coconut is also important in pharmaceuticals and cosmetics [2]. VCO has antimicrobial and, antiviral properties retaining vitamins and antioxidants [3]. With traditional usage for over three decades, VCO is often used for enhancing hair beauty and growth, moisturizing the skin, and, treating diarrhea and skin inflammations as well as other minor diseases [4]. The process of obtaining coconut oil from coconut milk typically entails employing heating methods, wherein the coconut milk is subjected to specific temperatures and durations, resulting in the eventual production of coconut oil [5]. Other methods for producing VCO are wet extraction, centrifugation, and boiling [6]. Wet extraction or centrifugation works by separating the coconut milk into different components by gravitational force while the boiling method entails simply boiling the coconut milk to evaporate the water until it is left with only the proteins and oil [6].

However, there are several disadvantages of using these techniques including being costly in terms of money, energy, and time [7]. Additionally, elevated temperatures can potentially reduce the quantity and quality of the coconut oil [8]. Moreover, the color and composition of the oil may be influenced by high temperatures [2]. To overcome these problems, the fermentation technique can be used. This involves fermenting the coconut milk to separate the oil and

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protein components by introducing microorganisms, typically yeast [9]. In this current study, the chosen yeast strains namely tapai yeast, bread yeast, and tempeh yeast were subjected to varying fermentation durations namely 24, 36, and 48 hours, respectively. As a result of this fermentation, a tri-layered composition emerges. The uppermost layer is rich in VCO, representing the desired end product. The middle layer is characterized by its protein content, while the bottom layer consists of water [5]. This innovative approach not only addresses the challenges associated with high temperatures affecting oil quantity and quality but also introduces a method that allows for nuanced control of the fermentation process to tailor the coconut oil production according to specific parameters [1].

The amount of iodine in VCO is actuated by the number of unsaturated fatty acids [10]. This is because the lower the iodine number in the VCO, the lower the saturation of the FFAs. Apart from that, the number of FFAs which is expressed as % FFA is important to conclude the quality of the VCO [7]. Overall, this research is conducted to determine the type of yeast that will produce the highest yield of coconut oil. The final product namely VCO is value added with greater quality, lower iodine number, and lower amount of FFAs, has a particular odor and is mainly colorless [7].

Meanwhile, biodiesel is defined as a long chain of fatty acids and mono-alkyl esters derived from vegetable oil such as coconut oil, corn oil, sunflower seed oil, palm oil and even used cooking oil and grease waste [8]. Biodiesel is called green diesel or renewable diesel as it is a product of vegetable oil conversion. Biodiesel has been experimented with as the future substitute for diesel which has various weaknesses as biodiesel proposes no toxicity, is biodegradable, and contributes less to pollution [11]. Biodiesel works the same as petroleum fuels which are used for diesel engines [12].

Biodiesel is a product of the transesterification of vegetables oil as well as glycerol as the by-product. Other methods of producing biodiesel are thermal cracking and micro-emulsion [12]. The transesterification of vegetable oil occurs with the aid of a catalyst namely a homogenous catalyst or heterogeneous catalyst [13]. The catalyst works as the accelerator of the reaction and enables the reduction of activation energy. Transesterification is a reversible reaction; it is termed as so because it produces ester compound at the end of the reaction. Transesterification also involves alcoholysis in which methanol is commonly used for the reaction. When methanol is used in an esterification reaction, the reaction is called methanolysis whereas if ethanol is used, the reaction is called ethanolysis [12]. The methanolysis reaction produces biodiesel namely fatty acid methyl ester (FAME) while the ethanolysis reaction produces biodiesel namely fatty acid ethyl ester (FAEE). In this present study, the focus is on the production of biodiesel from VCO produced from coconut milk using fermentation methods with different types of yeast [14].

EXPERIMENTAL

Chemicals and Materials

Chemicals used in this study are 96% ethanol (C_2H_5OH), methanol (CH_3OH), 0.1 M NaOH solution, 15% KI solution, 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solution, anhydrous sodium sulfate (Na_2SO_4), phenolphthalein indicator, hexane (C_6H_{14}), potassium hydroxide (KOH), coconut milk, bread yeast (Mauripan brand), tapai yeast, tempeh yeast (Raprima) and pineapple extract.

Preparation of Virgin Oil from Coconut Milk

The coconut milk was left for 8 hours at room temperature and the coconut cream was separated from the water [3]. After that, approximately 0.2 g of yeast was added and stirred. The four types of yeast used are bread yeast, tapai yeast, tempeh yeast, and bread yeast with pineapple extract. Three 250 mL beakers were set up for the bread yeast, tapai yeast, and tempeh yeast. Each beaker was added with 100 mL of coconut cream. Meanwhile, for the bread yeast with pineapple extract, 20 mL of pineapple extract was added along with 0.2 g bread yeast into 100 mL coconut cream in a 250 mL beaker. Each beaker was labeled for 18, 36, and 42 hours approximately. After each time of fermentation, the mixture formed 3 layers with VCO forming the first layer followed by protein and water [14]. Then, the mixture was centrifuged at 2700 rpm for 20 minutes. After that, the VCO layer was collected using a dropper and weighed to calculate the percentage yield. Next, the VCO was characterized using Fourier-Transform Infrared (FTIR) to analyze the functional group.

The yield of VCO was calculated using Equation 1 below [8].

$$\text{Yield of VCO (\%)} = \frac{Weight\ of\ VCO\ produced}{Initial\ weight\ of\ the\ raw\ materials\ used} \times 100\% \quad (1)$$

Transesterification of VCO into Biodiesel

About 5 g of VCO was weighed in an Erlenmeyer flask. Then, 38 mL of 96% ethanol was measured and put into the 250 mL volumetric flask; 0.5 wt% KOH was dissolved completely before being transferred into the Erlenmeyer flask containing the VCO. The ratio of the VCO and ethanol was 1:6. After that, the Erlenmeyer flask was put into the sonicator for 7 minutes. Based on research, the best time for transesterification is 7 minutes to produce the highest quantity of ethyl ester [15]. Therefore, the transesterification was fixed for all reactions at 7 minutes as the focus is to produce ethyl ester. After the sonication ended, the mixture was dried using anhydrous sodium sulfate and then filtered using a filter funnel. Next, the mixture was subjected to rotary vapor to erase all the untreated ethanol [12]. The mixture was then centrifuged at 2700 rpm for 3 mins and the result was two layers of biodiesel (ethyl ester) and glycerol. The biodiesel at the top layer was collected using a dropper and transferred into the sample bottle. The steps were repeated using 0.7 wt% and 0.9 wt% of KOH catalyst. The yield of biodiesel was calculated using Equation 2 [16].

Characterization Methods

Organolepstic test. The physical quality of the VCO was observed in terms of color, odor, and appearance. These parameters were analyzed using a numeric scale of 1 to 7 as shown in Table 1 [2].

Table 1. The numeric scale and the definition.

Numeric scale	Definition
1	Very bad
2	Bad
3	Rather bad
4	Neutral
5	Rather good
6	Good
7	Very good

Free fatty acid content. About 10 g of VCO was added to an Erlenmeyer flask [9]. Then, 50 mL of 95% ethyl alcohol was added and the mixture was heated for 10 mins [1]. After that, the mixture was left to cool and a phenolphthalein indicator was added. The mixture was then titrated using a previously standardized 0.1 M NaOH solution. The titration was discontinued when the pink color appeared and remained for 30 seconds. The formula for calculating the FFA value is shown in Equation 3.

Iodine value (IV). The IV indicates how unsaturated the fatty acids are in the oil and fats. To determine the iodine levels, a sample of VCO produced through fermentation using tempeh, tapai, and bread yeast was examined. The MPOB Test Procedures p3.2:2004 were followed in determining the iodine readings [17]. Accordingly, 20 ml of a solvent (1:1 cyclohexane: glacial acetic acid) and 20 g of completely melted and homogenized sample was weighed into a conical glass flask with a circular glass stopper. The test component was removed to create a blank test. Once the reaction was complete, 150 ml of water and 20 ml of potassium iodide were added. Following that, a normal sodium thiosulphate solution was used to titrate the solution. The blank test was prepared and titrated in the same way. Calculation of the IV was carried out and the IV was expressed as g of I_2 / 100 g of oil as shown in Equation 4.

$$IV = \frac{12.69\, C\,(V_1 - V_2)}{M} \quad (4)$$

C is the concentration of sodium thiosulphate.

V_1 is the volume, in milliliters, of sodium thiosulphate solution used in blank test.

V_2 is the volume, in milliliters, of sodium thiosulphate solution used in the determination of sample.

M is the mass, in grams, of the sample.

Density of VCO, glycerol, and biodiesel. A sample bottle was washed, dried and weighed. Then, the bottle was filled with 1 mL of samples and weighed [18]. The density of VCO, glycerol, and biodiesel were calculated using Equation 5 below [19].

$$\text{Density of VCO} = \frac{Mass\ of\ VCO\ (g)}{Volume\ of\ VCO\ (mL)} \quad (5)$$

Fourier Transform Infrared Analysis. The VCO, glycerol and biodiesel were characterized using Fourier-Transform Infrared Attenuated Total Reflectance (FTIR-ATR) spectroscopy (Perkin Elmer Spectrum 400 FTIR Spectrometer) at a resolution of 4 cm^{-1} and the spectra were obtained between 4000 – 650 cm^{-1}.

$$\text{Yield of Biodiesel (\%)} = \frac{Weight\ of\ biodiesel\ produced\ (g)}{Weight\ of\ VCO\ (g)} \times 100\% \quad (2)$$

$$\text{FFA as lauric (\%)} = \frac{Weight\ of\ VCO\ producedmL\ of\ alkali\ x\ M\ x\ 20.0}{Mass{,}g\ of\ test\ portion} \ldots \quad (3)$$

Table 2. Organoleptic test on VCO from fermentation with different types of yeast.

Type of yeast	Parameters	Scale	Observations
Bread yeast	Colour	7	Colourless VCO was observed.
	Odour	6	Slightly coconut scented
	Appearance	7	High viscosity
Tapai yeast	Colour	6	Colourless
	Odour	6	Slightly coconut scented
	Appearance	7	High viscosity
Tempeh yeast	Colour	7	Colourless
	Odour	6	Slightly coconut scented
	Appearance	7	High viscosity
Bread yeast with the addition of pineapple extract	Colour	3	The colour of VCO appeared yellowish
	Odour	2	Strong pineapple scented
	Appearance	6	High viscosity

RESULTS AND DISCUSSION

Organoleptic test of VCO. The organoleptic test was conducted on all of the VCO samples as shown in Table 2. From the test, all of the VCO samples were colorless, had a coconut odor, and were viscous except for the VCO fermented with bread yeast with addition of pineapple extract, which was yellow and gave a pineapple odor as pineapple extract contains a strong pineapple scent and yellow color. However, the VCO was viscous like the other VCO samples.

Yield percentage of VCO. Table 3 shows the yield percentage of VCO produced by fermentation using different types of yeast. The yield percentage of VCO was calculated using Equation 1. From the data, it shows that the yield percentage of VCO decreases with the additional hours for all fermentation. After 18 hours of fermentation, the bread yeast resulted in a yield of 27.93%. In comparison, the tapai yeast fermentation recorded a slightly higher yield of 28.76%, while the tempeh yeast fermentation exhibited a lower yield of 13.81%. In the 18-hour fermentation period, the highest recorded yield was from the tapai yeast fermentation, followed by bread yeast, bread yeast with added pineapple extract, and tempeh yeast. Specifically, the percentages were 28.76%, 27.93%, 20.06%, and 13.81%, respectively. Moving to the 36-hour fermentation period, the bread yeast exhibited a yield of 3.68%. In contrast, the tapai yeast recorded a yield of 1.06%, while the tempeh yeast showed a higher yield of 8.74%. The bread yeast fermentation with added pineapple extract resulted in a yield of 7.33%. These results suggest variations in yield percentages based on different yeast types and fermentation durations, emphasizing the impact of these factors on the coconut oil production process.

Table 3. The yield percentage of VCO produced by fermentation using different types of yeast.

Type of yeast	Percentage yield of VCO (%)		
	18 hrs	36 hrs	42 hrs
Bread yeast	27.93	3.68	1.20
Tapai yeast	28.76	1.06	0
Tempeh yeast	13.81	8.74	3.74
Bread yeast with pineapple extract addition	20.06	7.33	3.32

Table 4. The yield percentage of biodiesel and glycerol

Wt% KOH / time (mins)	Percentage yield (%)		
	0.3 / 7	**0.5 / 7**	**0.7 / 7**
Biodiesel	81.90	74.62	72.80
Glycerol	34.00	40.00	50.00

The observed trend in the VCO yield percentages suggests that the fermentation process with tempeh yeast is particularly effective for maximizing oil production, even at an extended duration of 42 hours. Interestingly, bread yeast with added pineapple extract also demonstrated notable VCO production, surpassing bread yeast alone. This may indicate a synergistic effect between bread yeast and pineapple extract, contributing to enhanced oil yield [5]. Pineapple extract contains bromelain, an enzyme that can break down cell walls and aid in the release of oils from plant materials. When combined with yeast, which can also produce enzymes that break down cellulosic material, there can be a combined enzymatic action that enhances oil extraction efficiency [5]. In contrast, the fermentation with tapai yeast showed zero yield percentage at 42 hours, reinforcing the notion that the active oil production primarily occurred in the initial 18 hours. The slower oil production in the subsequent fermentation stages could be attributed to the breakdown of protein compounds into VCO during the earlier phase [1]. These findings underscore the importance of yeast type, fermentation duration, and additional components like pineapple extract in influencing the yield and efficiency of VCO production from coconut milk.

Yield percentage of biodiesel. The VCO produced from the fermentation with tapai yeast was further used in transesterification reaction because it has the highest percentage yield and the highest FFAs value (%). The yield percentage of biodiesel and glycerol was calculated and presented in Table 4. Specifically, at 7 minutes of reaction time, the yield percentages for biodiesel and glycerol were determined for three different weight percentages of potassium hydroxide (KOH). For the transesterification with 0.3 wt% KOH at 7 minutes, the yield percentage was 81.90% for biodiesel and 34% for glycerol. In the case of 0.5 wt% KOH at 7 minutes, the yield percentages were 76.42% for biodiesel and 40% for glycerol. Lastly, for 0.7 wt% KOH at 7 minutes, the yield percentages were 72.80% for biodiesel and 50% for glycerol. This finding proves that the highest yield percentage of biodiesel occurs in the transesterification with 0.3 wt% KOH at 7 minutes, followed by 0.5 wt% KOH and 0.7 wt% KOH. Conversely, the highest yield percentage of glycerol was observed in the transesterification with 0.7 wt% KOH at 7 minutes, followed by 0.5 wt% KOH and 0.3% KOH. This result might be attributed to the fact that a higher weight percentage of KOH catalyst may lead to saponification due to lower acidity and an increase in hydroxide ions in ethanol. This chemical process, in turn, influences the yield percentages of biodiesel and glycerol during transesterification which is in agreement with other studies [16].

Density of VCO, biodiesel, and glycerol. The density of VCO produced is shown in Table 5. The density of VCO from the fermentation with bread yeast was 0.865 g/mL. Meanwhile, the density of VCO from the fermentation with tapai yeast and tempeh yeast was 0.934 g/mL and 0.869 g/mL respectively. Lastly, the density of VCO from the fermentation with bread yeast with the addition of pineapple extract was 0.876 g/mL. In increasing order of density, VCO from the fermentation with bread yeast has the lowest density value followed by the fermentation with tempeh yeast and fermentation with bread yeast with the addition of pineapple extract. The VCO with the highest density value was the one fermented with tapai yeast. The average density of the VCO was 0.886 g/mL which falls within the standard of density value based on SNI 7381: 2008 which is between 0.86 g/mL and 0.91 g/mL indicating that the iodine value in all of the VCO produced was in a good amount except for the VCO produced from fermentation with tapai yeast.

Table 5. Density of VCO produced by fermentation with different types of yeast.

Type of yeast	Density (g/mL)
Bread yeast	0.865
Tapai yeast	0.934
Tempeh yeast	0.869
Bread yeast with addition pineapple extract	0.876

The iodine value indicates the amount of unsaturated fatty acids constituted in the oil. Unsaturated fatty acids can lead to oxidation of oil which can cause the oil to go rancid. Meanwhile, the density of VCO determined the viscosity and texture of the oil [1]. Therefore, the iodine value should be within the standard to conclude that it has good quality. The high density of VCO produced may be affected by the presence of water contents and yeast or other compounds that are present during the fermentation of coconut milk [1].

The density of the biodiesel and glycerol produced by each transesterification were calculated and shown in Table 6. The density of biodiesel produced by transesterification with 0.3 wt% KOH was 0.9100 g/mL, whereas the glycerol produced from the same transesterification has a density of 1.0002 g/mL. Meanwhile, for the transesterification with 0.5 wt% KOH, the density of the diesel produced was 1.1002 g/mL and the density for the glycerol was 0.9879 g/mL. For the last transesterification with 0.7 wt% KOH, the density of the biodiesel was 0.8706 g/mL and for the glycerol, 0.8875 g/mL. From the data, the biodiesel produced from the transesterification with 0.3 wt% KOH has the most ideal density. This is because biodiesel has a density of about 0.902 g/mL and the biodiesel from the first transesterification has the nearest density value compared to the others. Meanwhile, the glycerol produced from the transesterification with 0.3 wt% KOH also has the most ideal density among the other glycerols produced. This circumstance is because the density of the glycerol is the nearest to the actual density value which is 1.26 g/mL. The biodiesel produced from transesterification with 0.3 wt% of KOH will give the best engine performance compared to the other biodiesel produced. This is because, when the density is too high, the fluid becomes too thick for the engines to settle and a longer time is needed for suspension. Meanwhile, when the density is too low, the fluid will become thinner and the efficiency of the engine will become lower [20].

Iodine test. The iodine value of VCO is shown in Table 7. The iodine value for VCO produced from the fermentation with bread yeast is 7.2, which is nearly similar to the iodine value recorded for both VCO produced from the fermentation with tapai yeast and tempeh yeast which is 7.1. All the values are within the range of the Asian and Pacific Coconut Community (APCC) standard, i.e., between 4.1 to 11 which indicates that the iodine content in the oil was in a good amount [21].

Table 6. Density of biodiesel and glycerol.

Type	0.3 wt% KOH	0.5 wt% KOH	0.7 wt% KOH
	Density (g/mL)		
Biodiesel	0.910	1.100	0.871
Glycerol	1.000	0.988	0.888

Table 7. The iodine value of VCO produced by fermentation with different types of yeast

Type of yeast	Iodine Value
Bread yeast	7.2
Tapai yeast	7.1
Tempeh yeast	7.1
Bread yeast with addition pineapple extract	7.2

Table 8. The FFAs and APCC composition of VCO produced by fermentation with different types of yeast

Type of yeast	FFAs Value (%)
Bread yeast	0.56
Tapai yeast	0.80
Tempeh yeast	0.64
Bread yeast with addition pineapple extract	0.54

FFAs test. Table 8 shows the FFAs and APCC values of VCO produced by fermentation with different types of yeast. The FFAs test was conducted and the value was calculated for each VCO produced. VCO from the fermentation with bread yeast contains 0.56% FFAs. Meanwhile, VCO from the fermentation with tapai yeast and tempeh yeast contains 0.80% and 0.64% of FFAs respectively. Lastly, VCO from the fermentation with bread yeast with added pineapple extract contains 0.54% of FFAs. From the data, VCO from the fermentation with tapai yeast has the highest FFAs value followed by tempeh yeast, and bread yeast. Meanwhile, the VCO from the fermentation with bread yeast with the addition of pineapple extract has the lowest FFAs value. This is because the pineapple extract has a high concentration of protease enzymes that help lower water content during the fermentation, thus causing the VCO to have a low value of FFAs% [5]. Furthermore, the higher FFAs % value in VCO produced by fermentation with bread yeast, tapai yeast and tempeh yeast is due to the hydrolysis process that leads to higher water contents which causes the FFAs% value to be higher than that from the fermentation with bread yeast with the addition of pineapple extract [1]. The percentage of FFAs (C12:0) for VCO produced by fermentation with bread yeast, tapai yeast, tempeh and bread yeast with the addition of pineapple extract exceeded the standard values of APCC which is 0.2 [21]. This is due to the excessive amount of moisture present in the oil produced during the fermentation process, the moisture can be reduced by using the fermentation method stated by Susilowati (2019) with coal ash adsorbent [22].

Characterization

Fourier Transform Infrared Analysis

Figure 1 shows the FTIR spectrum of VCO produced from the fermentation of bread yeast, tapai yeast, tempeh yeast and bread yeast added with pineapple extract. All the spectrums show nearly similar wavenumbers with three important peaks indicating the functional groups in each spectrum. For all the spectrums, there is a couple of sp^3 C-H bond stretches at 2924 cm^{-1} and 2853 cm^{-1}. Besides that, there is a strong intensity of C=O bond stretch at 1745 cm^{-1} and a narrow C-O bond stretch at 1160 cm^{-1} which indicate the presence of lauric acid and fatty acids in the form of ester [23]. The analysis of the VCO functional group and wavenumber are summarized in Table 9.

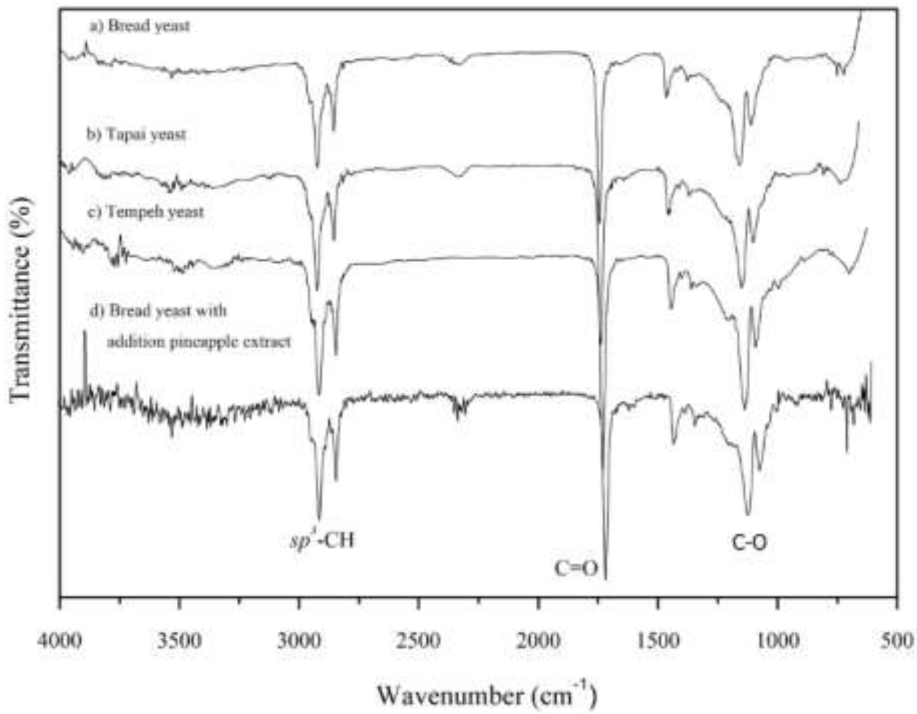

Figure 1. FTIR spectrum of VCO produced from the fermentation of a) bread yeast; b) tapai yeast; c) tempeh yeast, and d) bread yeast with the addition of pineapple extract.

Table 9. FTIR analysis of VCO produced by fermentation using bread yeast, tapai yeast, tempeh yeast, and bread yeast with addition pineapple extract.

Functional group	Wavenumber (cm^{-1})			
	Bread yeast	**Tapai yeast**	**Tempeh yeast**	**Bread yeast with addition pineapple extract**
Alkanes, sp^3 C-H	2924, 2853	2924, 2853	2923, 2853	2925, 2857
Ester, C=O	1745	1745	1746	1745
Ester, C-O	1160	1159	1157	1158

Figure 2 shows the FTIR spectrum of biodiesel produced by esterification using different wt% of KOH. All of the three spectrums also show nearly the same bond stretch and wavenumber. Three important peaks indicate that this compound is biodiesel. First, there are a couple of sp^3 C-H bond stretches at 2952 cm^{-1} and 2856 cm^{-1}. Then, at wavenumber 1740 cm^{-1}, there is a sharp and strong intensity of stretch which indicates the presence of a C=O bond in the compound. Lastly, there is a narrow stretch at 1176 cm^{-1} which indicates the C-O bond. The carbonyl compounds formed are triglyceride which is the main compound in vegetable oil, consisting of glycerol groups and free fatty acids that are mostly present in the trace form of esters [24]. The FTIR analysis of biodiesel is summarized in Table 10.

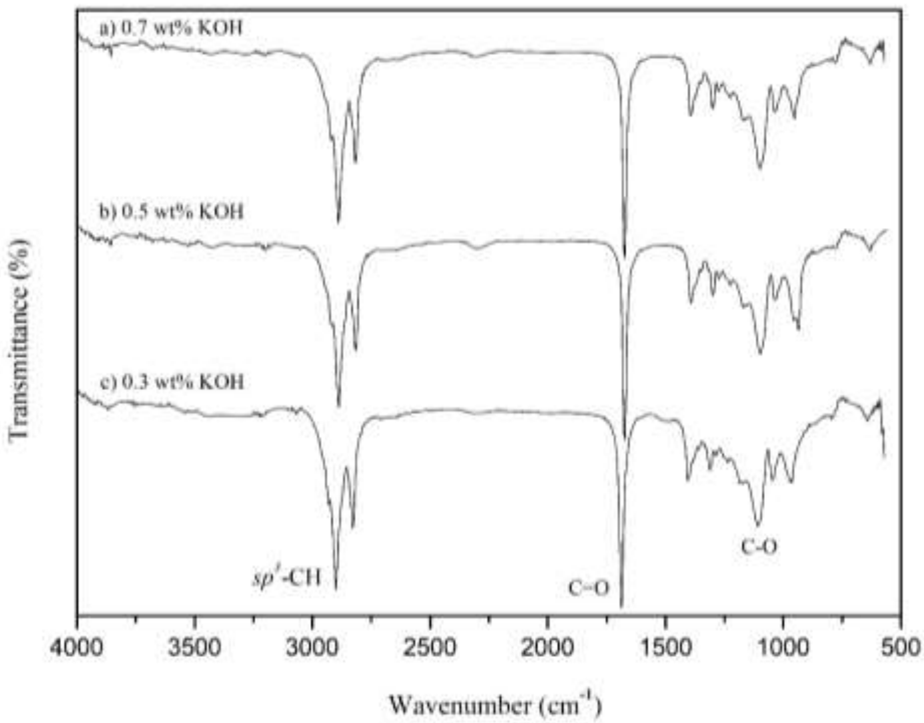

Figure 2. FTIR spectrum of biodiesel produced by esterification with a) 0.7 wt% KOH; b) 0.5 wt% KOH, and c). 0.3 wt% KOH.

Table 10. FTIR analysis of biodiesel.

Functional group	Wavenumber (cm^{-1})		
	0.3 wt% KOH	**0.5 wt% KOH**	**0.7 wt% KOH**
Alkanes, sp^3 C-H	2925, 2856	2925, 2855	2925, 2856
Ester, C=O	1740	1739	1739
Ester, C-O	1176	1177	1177

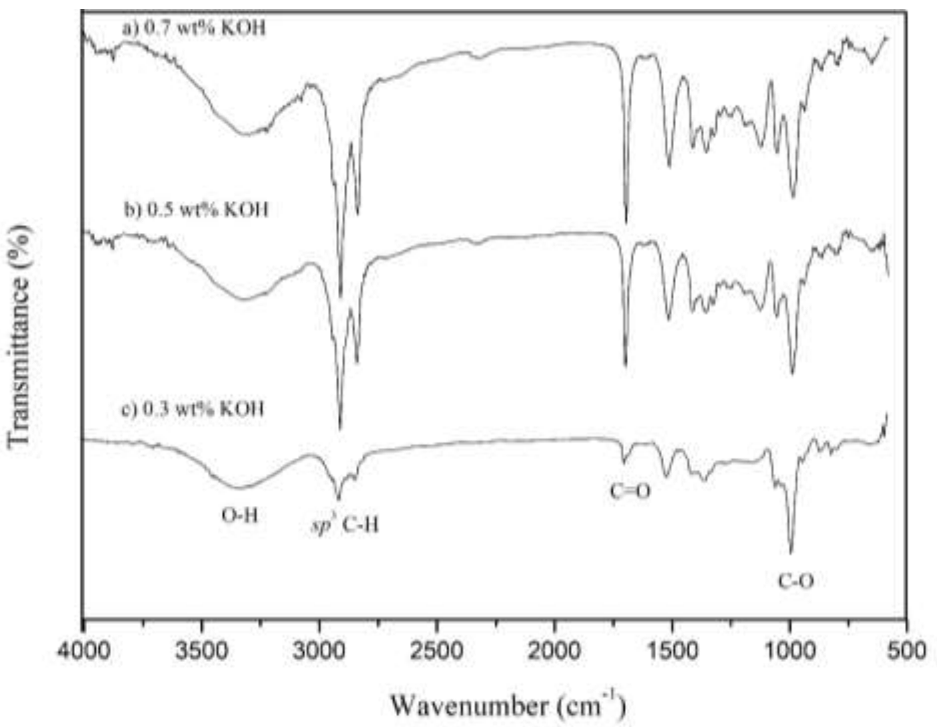

Figure 3. FTIR spectrum of glycerol produced by esterification with a) 0.7 wt% KOH; b) 0.5 wt% KOH, and c) 0.3 wt% KOH.

Table 11. FTIR analysis of glycerol.

Functional group	**Wavenumber (cm^{-1})**		
	0.3 wt% KOH	**0.5 wt% KOH**	**0.7 wt% KOH**
Alcohol, O-H	3319	3320	3306
Alkanes, sp^3 C-H	2924, 2857	2925, 2855	2925, 2856
Ester, C=O	1738	1740	1740
Ester, C-O	1047	1047	1047

Figure 3 shows the FTIR spectrum of glycerol. All the spectrum also shows similar adsorption and wavenumber which highlight four important peaks. The first peak is the broad adsorption at 3319 cm^{-1} which indicates the presence of an O-H bond in the compound structure. Next, there is a sharp and strong intensity of stretch at 2924 cm^{-1} and 2857 cm^{-1} which indicates the sp^3 C-H bond. Besides that, there is a sharp peak at 1738 cm^{-1} which indicates the C=O bond. Lastly, there is also a sharp peak at 1047 cm^{-1} which indicates the C-O bond. Both bonds show the presence of fatty acids in the form of esters [23]. The presence of different percentages of KOH might induce changes in the local molecular environment or conformation of glycerol molecules, affecting the intensity of certain vibrational modes in the FTIR spectrum. The FTIR analysis of glycerol is summarized in Table 11.

CONCLUSION

In conclusion, the set objectives were achieved successfully across various stages of the study. Fermentation using tapai yeast produced the highest amount of VCO and the highest amount of FFA. Thus, using tapai yeast for fermentation for further transesterification is the best as more ethyl ester can be produced. The extraction of VCO through fermentation by employing different yeast types, demonstrated effectiveness. Additionally, the successful production of biodiesel through transesterification with ethanol in the presence of a KOH catalyst was also achieved. Notably, the coconut milk fermentation with tapai yeast at 18 hours demonstrated optimal conditions for VCO production, yielding the highest percentage (28.76%) and the highest FFA value (0.8%). Consequently, tapai yeast fermentation emerges as the preferred method for biodiesel production through transesterification. Furthermore, transesterification of VCO with 0.3 wt% KOH at 7 minutes exhibited the highest biodiesel yield (81.9%) and solidified it as the preferred configuration for the transesterification process. The comprehensive success of these processes underscores their viability for sustainable and efficient biofuel production.

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