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Green Technology on the Virgin Coconut Oil Production Using Enzyme from Pineapple Waste

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ABSTRACT

Virgin coconut oil (VCO) is widely used in the pharmaceutical and cosmetic industries. The high lauric acid content is very beneficial in the pharmaceutical field, such as antiviral and antibiotics. This study proceeded with VCO production using an enzymatic way that was efficiently conducted and environmentally friendly. The main materials used in this research included coconut (*Cocos nucifera* L) and pineapple (*Ananas comosus* L). This research aims to identify the enzymatic process of VCO production by using pineapple waste, including pineapple crowns, pineapple fruit skins, pineapple leaves, and pineapple trunks. The pineapple waste contains the enzyme bromelain to break down protein emulators in coconut milk cream. The production was conducted in a 250 mL measuring cylinder glass and incubated in the water bath at 30°C, 50°C, and 80°C. The progress of VCO production was observed every 1(one) h for 3 h long experiment. The ratio between the coconut cream and fresh pineapple waste was 1:1 until 9:1. Based on the experiment data with variations in substrate volume and temperature, the optimal VCO formation was obtained at 50°C with a ratio between the substrate and enzyme material of 9:1. The VCO was produced at an average of 57 mL from 200 mL of the initial volume of coconut cream. VCO quality was evaluated as water content, free fatty acid concentration, and saponification numbers. Based on the evaluation results, VCO's quality met SNI's standard and Codex Alimentarius Commission.

Keywords: Enzymatic, Pineapple crown extract, Virgin Coconut Oil

INTRODUCTION

Virgin Coconut Oil (VCO) contains lauric acid, which belongs to the medium-chain fatty acids (MCFA). This content makes VCO useful in the pharmaceutical field, such as in treating hypercholesterolemia, diabetes, and hypertension. It also has activities as an antibiotic, antiviral, and cosmetic formulation purposes (Chinwong *et al*., 2017). VCO has the function of a moisturizer in cosmetics. Besides, it can also be used as a carrier material in medicinal preparations (Agero and Verallo-Rowell, 2004; Lima and Block, 2019).

VCO's high demand encourages the researcher to conduct many types of research regarding VCO production. It was reported that VCO production could be conducted using various methods, which were processed by minimizing the heating. The VCO production is divided into three methods: physical, chemical, and enzymatic. The physical method is a production involving centrifugation or low-temperature heating. The chemical method involves acidification or chemical stimulation. Meanwhile, an enzymatic method involves protein degrading enzymes (Anzaku *et al*., 2017; da Silva Lima and Block, 2019; Putri, 2014; Rohyami *et al*., 2017). Generally, the VCO is produced by furtherly processing coconut milk to extract oil (Lima and Block, 2019; Hidayati, 2010; Marina *et al*., 2009; Prapun, *et al*., 2016; Putri, 2014; Raghavendra and Raghavarao, 2010; Raya *et al*.,

2009; Ricochon and Muniglia, 2010; Rohyami *et al*., 2017). Coconut milk or *Santan* is an emulsion consisting of aqueous and oil phases that are not mixed as they have an emulator in the form of a protein as a stabilizer (Masyithah, 2017). The emulsifying agent, which underwent a breaking down process using a proteolytic enzyme, was reported as an environmental process (Harimurti *et al*., 2020). It is a non-toxic ingredient, reactions of active, accelerating agents at low concentrations (Kumaunang and Tabaga, 2019).

The proteolytic enzyme can be found in several raw materials, such as pineapple. The proteolytic enzyme in the pineapple is called bromelain. The bromelain enzyme can be obtained from the skin, stems, leaves, fruit, weevils, and even the crown of pineapple flowers in different amounts (Kumaunang and Tabaga, 2019). One pineapple only has 53% for consumption, while the rest is discarded, turning into waste that cannot be consumed (Rulianah, 2002). Based on previous research, all parts of pineapple contain the bromelain enzyme; therefore, all these parts can be utilized, especially concerning the bromelain content. Based on the increase in VCO demand and the abundance of raw material, i.e., coconut and pineapple wastes, this research aims to determine the best condition and raw material for production. This paper specifically reported the study of VCO production by utilizing pineapple waste. The preparation of raw material, the production process, and VCO production kinetic will be explained. This report may be useful for the upcoming environmentally VCO production to fulfill the high demand for VCO.

Figure 1. Raw materials used for VCO production

MATERIAL AND METHOD

Grated coconut meat, pineapple crown, pineapple leaves, pineapple trunk, and pineapple skin fruit were obtained from the Mangunan, Bantul, Special Region of Yogyakarta. The grated coconut meat and parts of pineapples used in the research (Figure 1). Analytical grade of Hydrochloric acid (HCl), analytical grade of Sodium Hydroxide (NaOH), analytical grade of Oxalic acid, and analytical grade of Potassium Hydroxide (KOH) were purchased from Merck Germany. Distilled water, alcohol 96%, and phenolphthalein indicator were obtained from Brataco Indonesia.

VCO production

Preparation of the material for VCO production started with preparing the coconut cream. The coconut cream was made by preparing 3 kg of grated coconut meat and adding 3 liters of distilled water (1:1). They were squeezed and filtered. Coconut milk was then left for one h to form two layers. The top layer is called cream, and the bottom layer is called skim. The coconut milk skim was removed, and the coconut milk cream was taken as the material for VCO production. The pineapple waste extract was carried out while preparing the coconut cream. Pineapple waste extracts were prepared by extracting clean pineapple waste that had been washed and cut into small pieces. The pineapple waste was then blended with distilled water at the ratio between pineapple waste and water, as follows: pineapple crown (1:2); pineapple skin fruit (1:1); pineapple leaves (1:2); and pineapple trunk (1:1), respectively. Furthermore, the mixture was squeezed to collect the filtrate and note the volume.

VCO was made by mixing 20 mL of pineapple waste extract and the coconut cream volume variation in a 250 mL measuring cylinder glass. The coconut cream volumes used were 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mL. Each combination of the waste extract and coconut cream was then incubated at 30°C, 50°C, and 80°C for 3 h. Three different layers were obtained during the incubation, including *blondo*, oil, and water. That middle layer is called the VCO. The development of VCO during the incubation was noted to study the rate of VCO production. The VCO was then collected and evaluated to identify its quality. The organoleptic test, water content, free fatty acid concentration, and saponification number were evaluated for the VCO produced to evaluate VCO's quality.

Table I. The K_m and V_{max} values for the Lineweaver-Burk plot for the different enzyme sources and temperature.

Temperature		**Y= bx + a**		**V_{max}**	**K_m**
		a	**b**	**1/a**	**b x V_{max}**
Pineapple crown	30^0C	-1.35	143.17	-0.74	-10.49
	50^0C	-0.35	50.83	-2.85	-145.22
	80^0C	-4.58	326.78	-0.22	-71.35
Pineapple skin fruit	30^0C	-16.37	1456.40	-0.06	-88.90
	50^0C	0.29	1.00	3.40	3.41
	80^0C	-2.07	217.16	-0.48	-104.90
Pineapple leaves	30^0C	-0.53	50.43	-1.89	-95.15
	50^0C	-0.11	33.15	-9.09	-301.36
	80^0C	-0.65	72.67	1.53	-11.80
Pineapple trunk	30^0C	-2.71	269.61	-0.37	-99.49
	50^0C	-1.29	122.74	-0.77	-95.15
	80^0C	-2.58	246.60	-0.39	-95.58

Analysis

Organoleptic analysis

The VCO was physically analyzed using the organoleptic method by testing it with senses, including the sense of smell (nose), sense of sight (eyes), and sense of touch (fingers). Physical analysis carried out included its smell, its texture, and its color. An organoleptic test was utilized to determine the appearance of the VCO produced.

Water content

A 2g VCO was weighed in a porcelain cup, then heated in an oven at 105°C for 5h. The VCO was weighed and reheated in the oven for 1 h. After that, VCO's weighing would be done if a constant weight was obtained (Yang *et al*., 2020).

Free fatty acid numbers

A 10g VCO was weighed and put into a 250mL Erlenmeyer flask. After that, 95% of 50 mL neutral alcohol was added. It was then heated until boiling for 10 min with an electric stove and was given three drops of PP indicator. Furthermore, the solution was titrated with 0.1 N of KOH until the solution turned pink (violet) and did not disappear for ±30s (Prapun, R.; Cheetangdee, N.; Udomrati, 2016). KOH that was used was standardized using a 0.5 N HCl standard solution.

Saponification numbers

A 3g VCO sample was weighed in a 250mL Erlenmeyer flask. 50 mL of ethanolic KOH was then put into it. The Erlenmeyer flask was connected to an upright chiller and boiled for 30 min. After that, the solution was chilled and added with three drops of phenolphthalein indicator, and it was then titrated with 0.5N of HCl until the pink color disappeared (Harni & Putri, 2014).

Enzyme Kinetics

Enzyme activities could be identified by determining the rate of VCO formation (V) obtained from linear regression based on research data and substrate concentration. The Lineweaver-Burk plot was then made to determine the value of K_m and V_{max} (Lai *et al*., 2014).

RESULT AND DISCUSSION

VCO production

VCO production in this study was conducted using the enzymatic method. The raw materials used were coconut cream and bromelain enzyme derived from the pineapple waste, such as pineapple crown, pineapple skin fruit, pineapple leaves, and pineapple trunk. According to previous study, each enzyme can work optimally at a specific temperature range. The reaction will slowly occur at low temperatures, while at high temperatures, the reaction can occur quickly. However, using temperatures exceeding the optimum limit can cause the enzymes to experience denaturation, so the reaction rate decreases (Elias *et al*., 2014).

The VCO production in this study was carried out using three temperature variations: 30°C, 50°C, and 80°C. The volume of pineapple waste extract was kept constant at 20 mL in every production set, while the amount of the substrate varied, namely, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mL.

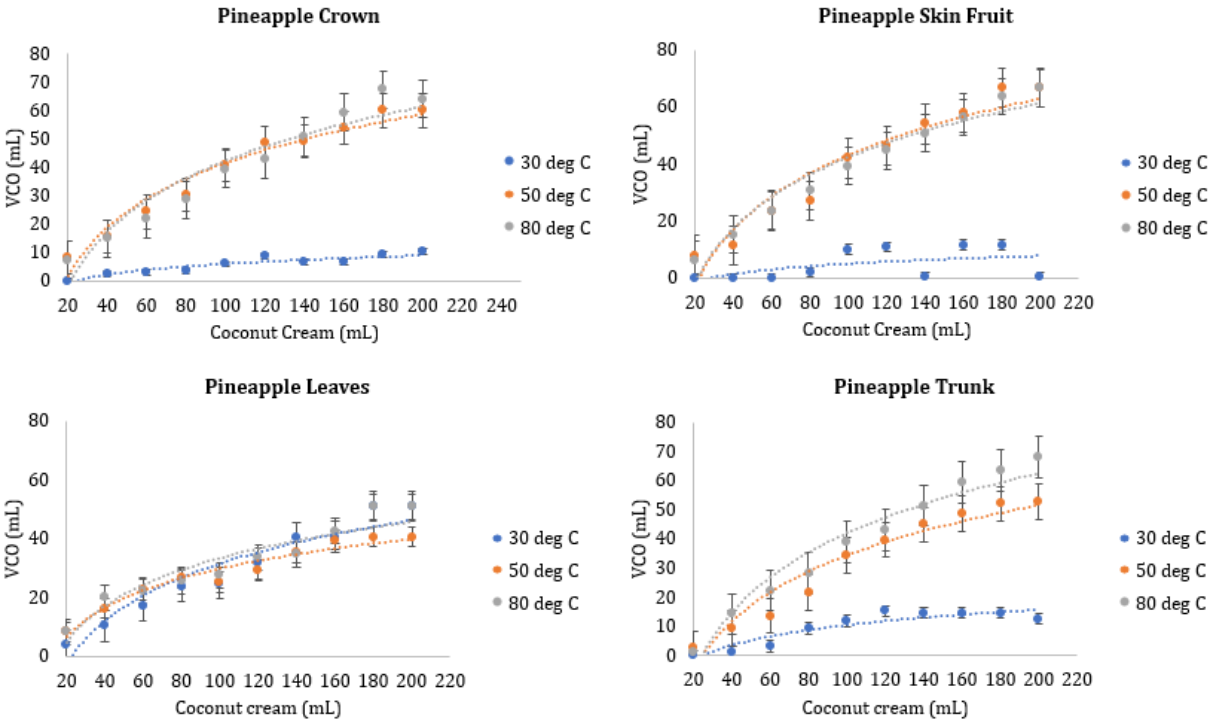

Figure 2. The average acquisition of VCO for four (4) kinds of pineapple waste at the same amount of pineapple extract waste (20 mL) and different incubation temperatures

Based on data from the National Center for Biotechnology Information, the bromelain enzyme worked optimally in the temperature range of 50-60°C and would experience enzyme inactivation at temperatures over 70°C (Herdyastuti, 2006). Therefore, the production was conducted at three different temperatures to identify the best temperature for production. The trendline for each temperature can be seen in a graph of the average acquisition of VCO (Figure 2). When the three profiles were compared, (Figure 2), a significant difference in VCO produced a trendline between the graphs at 30°C, 50°C, and 80°C. The trend of VCO production of 30°C looked much lower than the 50°C and 80°C, except for the production with pineapple leaves extract. When the 30°C was used, the production rate was similar to the temperatures of 50°C and 80°C. The graft of 50°C and 80°C for production using pineapple crown, pineapple skin fruit, and pineapple trunk had overlapped trend lines. It indicated that the bromelain enzyme from pineapple waste worked optimally at a temperature of 50°C, while at a temperature of 30°C or lower, the reaction would take slowly, and the bromelain enzyme became inactivated at a temperature of 80°C. VCO formation at 80°C was considerable due to the heating that caused the protein emulator to be damaged or denatured so that the oil inside coconut milk was extracted (Wuryanti, 2004). These results align with the data that has been reported by Herdyastuti (2006) on the isolation and characterization of raw bromelain from pineapple trunks. It was mentioned that the temperature for bromelain worked optimally at 55°C (Herdyastuti, 2006).

The VCO's acquisition volume at temperatures of 50°C and 80°C had a coinciding trendline for production using the crown, skin fruit, and trunk. The coinciding trendline also happened for production using pineapple leaves between the temperature of 30°C and 80°C. Therefore, a statistical test analysis was conducted to determine the significance of the VCO produced between the two temperatures. The statistical test was carried out through an Independent sample t-test. The independent sample t-test showed a significance p-value greater than 0.05 ($p>0.05$), indicating no significant difference between the VCO produced at 50°C and 80°C for the production using the crown, skin fruit, and trunk. Based on the results, the temperature of

50°C was optimal for VCO formation according to the enzyme activity. A higher temperature of 80°C gave a similar result to 50°C, based on the heating effect. The heating will break down the protein emulator of coconut cream (Raghavendra and Raghavarao, 2010). This result aligns with previous research about the activity of the bromelain enzyme that it will be inactive or denatured at 80°C (Herdyastuti, 2006). Meanwhile, the independent sample t-test was conducted for VCO production using pineapple leaves at a temperature of 30°C and 80°C since the trendline coincided. There was also no significant difference between the temperature of 30°C and 80°C. Based on the evaluation of the enzymatic VCO production at varying bromelain enzyme sources, the best temperature was 50°C for pineapple trunk, pineapple skin fruit, and pineapple crown fruit. The VCO produced were 52 mL, 67 mL, and 60 mL from 200 mL of coconut cream. Meanwhile, for pineapple leave enzyme source, the best temperature was 30°C for the 51 mL VCO from 200 mL of coconut cream.

Quality analysis

The appearance or organoleptic observation and the quality of VCO produced during the study are summarized as follows.

Organoleptic observation

According to the Agency of Indonesian National Standard (SNI 7381: 2008), the VCO has no color (clear), has no taste, and has a distinctive and fragrant aroma (SNI, 2008). VCO's organoleptic test was conducted by testing the VCO based on the sense of touch, smell, and vision. Based on the Codex standard, the organoleptic test for virgin coconut oil must be free from foreign and rancid odor and taste (Commission Codex Alimentarius, 1995). The VCO in the experiment of this study smelled like coconut for all different products. The texture of the VCO was a slightly runny texture for all experiments. However, only the VCO, whose production used pineapple trunk, was colorless, while the other had a greenish color. VCO's greenish color was caused by the production process involving bromelain enzyme from the pineapple crown, pineapple skin fruit, and pineapple leaves. These enzyme sources contained chlorophyll, which gave the greenish color.

Water content

Water content was measured in the percentage of the amount of material evaporated by heating using an oven at a particular time and temperature. In this study, the gravimetric method tested the water content, where 2 g of VCO as a sample were put in the oven at 105°C for 5 h. Water content analysis was carried out three times for replication in this study. The VCO's water content produced using pineapple crown and pineapple skin fruit did not meet the standards of the Codex Alimentarius Commission, which was 0.2% at maximum. It could occur as the process of purification of VCO needed to be improved to remove the water content added during the process of coconut cream preparation.

Free fatty acid numbers

Free fatty acids could be formed due to hydrolysis reactions. The hydrolysis reaction in oil occurred due to the presence of water, in which fat was hydrolyzed and damaged. Free fatty acid levels were used as a benchmark for VCO quality. The lower levels of free fatty acids showed that VCO was resistant to rancidity. The presence of free fatty acids in VCO would result in an unpleasant smell and taste (Ghosh *et al*., 2016).

This study performed three replications to determine the levels of free fatty acids found in VCO. Based on VCO's Codex standard, the maximum free fatty acid concentration was 0.2% (Commission Codex Alimentarius, 1995). According to the APCC, the standard for maximum free fatty acid levels is 0.5%. Free fatty acid numbers higher than this standard were considered to occur due to the hydrolysis reaction accelerated by water in the VCO. Incorrect VCO storage for a long time could also be a factor in hydrolysis; thus, the number of free fatty acids increased (Mohammed *et al*., 2021).

Saponification numbers

Saponification can be conducted by adding a strong base such as KOH to allow the oil to undergo a hydrolysis reaction. Perfect hydrolysis can occur when the addition of KOH is carried out excessively. The saponification number is inversely proportional to the molecular weight of oil (Odoom & Edusei, 2015). The greater the meaning of the saponification number is, the smaller the molecular weight of the oil component will be. Small molecular weight oils have large short-chain fatty acids, making the oil more stable, durable, and not quickly rancid. Based on the result of this study, the average saponification number was 250.75. The result met the requirements of saponification numbers on VCO by APCC, which is between 250 and 260.

Kinetic study

The bromelain enzyme obtained from fresh extracts of the stem, fruit skin, crown, and leaves is a proteolytic enzyme that can catalyze the hydrolysis reaction of proteins (Resmi Mohan and Muralidharan, 2016). The main content in bromelain is a sulfhydryl protease enzyme that can hydrolyze the peptide bonds of emulator proteins into amino acids with small molecules (Esfandi *et al*., 2019; Shi *et al*., 2021).

Bromelain is found in old and young pineapples. However, the activity of the bromelain enzyme in younger pineapples is much higher than in older pineapples. Adding the bromelain enzyme to *Santan* cream can help break down proteins that function as emulsifiers in *Santan* to increase the VCO yield (Winarti, S., and Jariyah, 2007). Yield is the amount of VCO product divided by the amount of *Santan* cream used as a starting raw material. The yield value will be directly proportional to the amount of oil produced (Ghani *et al*., 2018). Based on the experimental data, a high yield was found when VCO's production was using the fresh crown and leaves extract. The crown's concentration and leave for making the bromelain extract were half compared to the skin fruit dan trunk. Surprisingly, the yield was similar to the other. The highest yields from different enzyme sources include crown, leaves, skin fruit, and trunk.

Enzymes are proteins that play a role in the catalysis of biochemical reactions inside and outside the cell. An enzyme is a globular molecule consisting of one or more polypeptide chains (Elias *et al*., 2014). Enzyme kinetic reaction is a branch of enzymology identifying factors that influence the speed of enzymatic reactions, such as substrate concentration. Substrate concentration can be used to identify an enzyme's reaction mechanism, namely, the stages of substrate binding by an enzyme or the release of its product (Andrić *et al*., 2010). In enzyme kinetic reactions, the plot of a relationship between substrate concentration (S) and reaction rate (V) is called plot Michaelis-Menten. This plot can determine the Michaelis-Menten constant (K_m), specific for the different enzymes. The K_m is a constant indicating the substrate concentration needed by an enzyme to reach half its maximum rate reaction (V_{max}) (Lai *et al*., 2014).

The curve of the Michaelis-Menten plot is written in equation 1. The linearization of equation one (1) can be seen in equation 2. Equation 2 visualizes the Lineweaver-Burk plots or multiple reciprocal plots. By using this plot, the K_m and V_{max} can be determined based on the experimental data (Lai *et al*., 2014).

$$V = V_{max}\frac{[S]}{[S]+K_m} \quad \text{.....(1)}$$

$$\frac{1}{V} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \text{.....(2)}$$

The substrate concentration influenced the rate of VCO formation catalyzed by the bromelain enzyme. When the substrate concentration increased and the other conditions were kept constant, the initial measured rate (V) would increase until V_{max}, or maximum rate was achieved (Figures 3, 4, 5, and 6, respectively). V value is directly proportional to the increase in substrate concentration (Lai *et al*., 2014). The reaction rate would continue to increase until the substrate saturated the enzyme. The increase in substrate concentration no longer influenced the reaction rate when the reaction reached its maximum value. It was regarding the substrate that had an excessive number of molecules over the number of enzyme molecules. The Michaelis-Menten constant or K_m value can be interpreted as a substrate concentration that produces half the maximum speed (Lai *et al*., 2014; Reuveni *et al*., 2014).

V_{max} and K_m values are parameters used in determining enzymatic kinetics. This parameter can be determined by a chart of the relationship between substrate concentration [S] and reaction speed (V). Determination of V_{max} value (maximum rate) and K_m value (Michaelis-Menten constant) at each temperature used the Lineweaver-Burk plot. V_{max} and K_m were determined using the equation obtained from the regression chart with axis x of 1/[S] and axis y of 1/V following the Lineweaver-Burk plot. Next, the calculation of V_{max} and K_m values was carried out using the regression equation y = ax + b, where a (slope) = K_m/V_{max} and b (intercept) = $1/V_{max}$.

The kinetic parameter (Table I) was determined from the plot of Lineweaver-Burk of experiment data for each different method and different temperature.

The values of K_m and V_{max} can be determined even though the graph shows a downward curve. The data obtained were biased and had negative results for K_m and V_{max}. The V_{max} and K_m could be determined only by the production using pineapple skin fruits. However, the R^2 of the equation to calculate the kinetic parameter was very low.

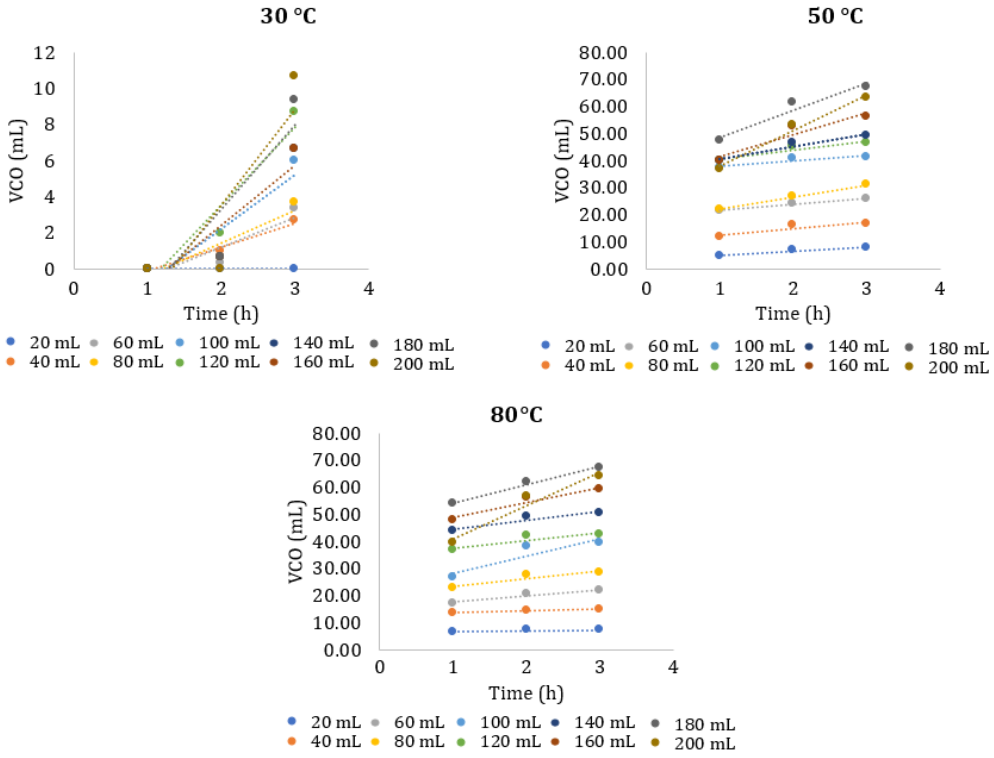

Figure 3. The rate of VCO production using pineapple crown at varying substrates and temperatures (30°C, 50°C, 80°C).

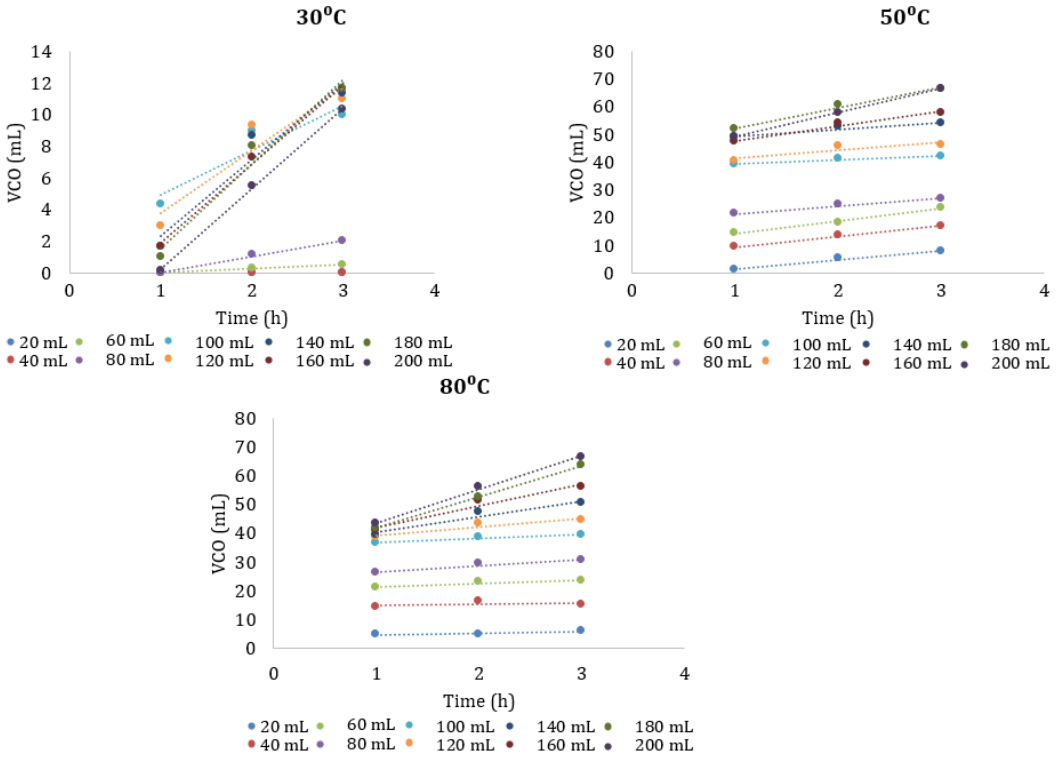

Figure 4. The rate of VCO production using pineapple skin fruit at varying substrates and temperatures (30°C, 50°C, 80°C)

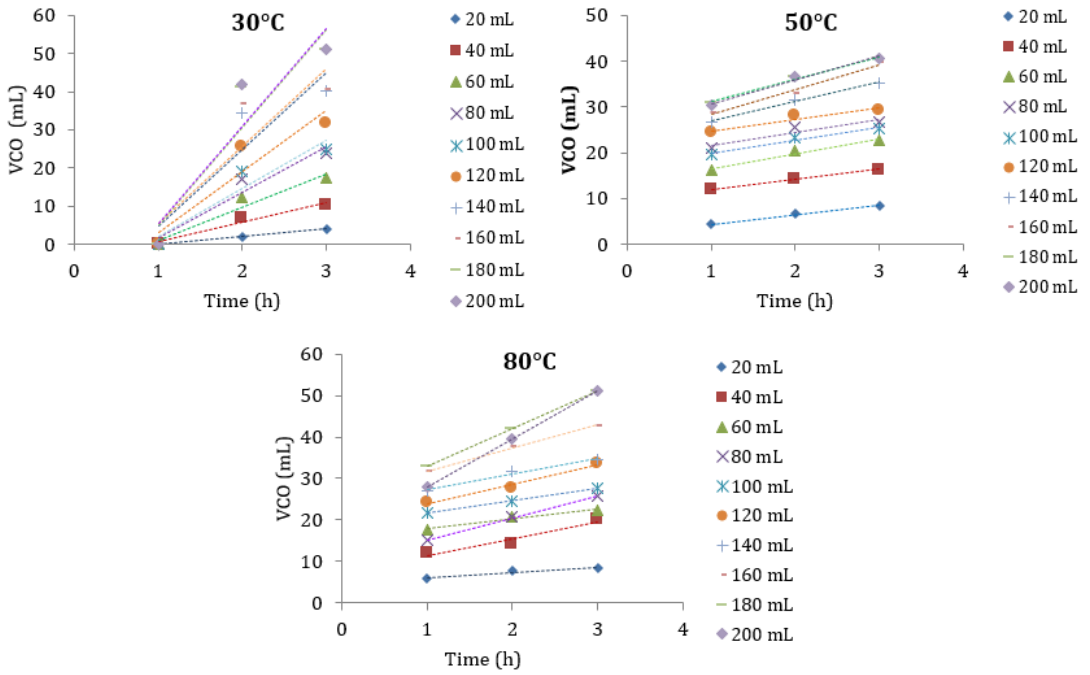

Figure 5. The rate of VCO production using pineapple leaves at varying substrates and temperatures (30ºC, 50ºC, 80ºC)

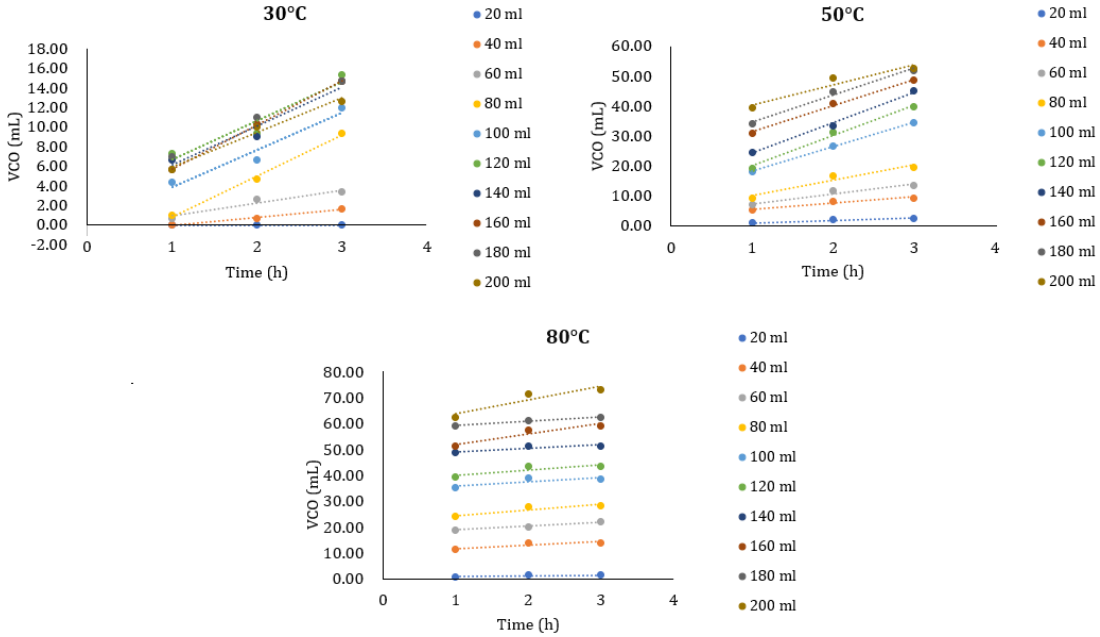

Figure 6. The rate of VCO production using pineapple trunk at varying substrate and temperature (30ºC, 50ºC, 80ºC)

Thus, it indicated that the Lineweaver-Burk plot could not be used to determine the kinetic parameter for enzymatic reaction in this VCO production. These might be related to the purity of the enzymes used. When the enzymes are used purely to allow the enzyme's active site, it can react better and increase its activity (Putra, 2009). Meanwhile, the bromelain enzyme extract from

pineapple waste used in this study was a crude extract that contained many other components besides the bromelain enzyme. It was most likely to cause the data obtained to be incorrect; thus, the K_m and V_{max} could not be determined (Fitrilia *et al*., 2017).

CONCLUSION

The pineapple waste, such as a crown, skin fruit, leaves, and trunk, could be utilized as the enzyme sources for VCO production with the limitation on VCO appearance except for VCO produced using pineapple trunk. The optimal VCO formation was obtained at a ratio between the coconut cream and pineapple waste juice at 9: 1. The best temperature for VCO production was 50°C when the production came to use crown, skin fruit, and trunk. However, in terms of the pineapple leaves, the best temperature for production was 30°C. The average VCO produced was 57 mL from 200 mL of the initial volume of coconut cream. Furthermore, VCO's quality, such as water content, free fatty acid concentration, and saponification numbers, met the standard of the Codex Alimentarius Commission.

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