Environmentally friendly production method of virgin coconut oil using enzymatic reaction

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Abstract. Virgin Coconut Oil (VCO) has been known in Indonesia since a decade ago. This oil is made from coconut milk using unheated method, such as enzymatic, physical, and chemical method. The VCO has been proofed to have many health benefits such as for treatment of hyper-cholesterol, diabetes, viral and bacterial infections. Also, the oil has been utilized in the cosmetics industry for beauty skin care. In the increase of VCO demand for pharmaceutical industry, this study was conducted to find the method for VCO production using an environmentally friendly process. The environmentally friendly process was conducted to maintain the sustainable environment. This paper deals with the enzymatic method of VCO production. The enzyme was obtained from fresh pineapple juice that is proofed contain bromelin. The enzyme will cleave the polypeptide bond of emulsifying agent in the coconut milk. Further, the oil will be separated and then collected as the VCO. During this work, the amount of the fresh pineapple juice varied to measure the rate of VOC production. Based on the observation during the production, it is found that the increase of fresh pineapple juice added increases the VCO production.

1. Introduction
Coconut oil has been known for centuries in human life. The type of coconut oil is divided into two, namely ordinary coconut oil and virgin coconut oil (VCO). Coconut oil is usually obtained from heating copra with high temperatures while VCO is obtained without heating [1]. VCO has advantages in helping the treatment process of disease. Active substance that helps in the treatment process is lauric acid [2]. Lauric acid content in VCO is able to cure a patient with high cholesterol, diabetes, stroke, hypertension, hepatitis, cancer, antibacterial and antiviral, and maintain healthy skin [3 – 5] In addition, lauric acid is also used as raw material for the cosmetics and pharmaceutical industries. According to the previous researches, the process of VCO production is divided into three methods, namely physical, chemical and enzymatic. Physical method is production involving low temperature heating and centrifugation. The chemical method is production involving acidification and stimulating method. While enzymatic method is production involving protein degrading enzyme [6 – 14].

The enzymatic method in VCO production uses coconut milk as the main ingredient and protease enzyme as a supporting ingredient. The enzyme will break down the coconut emulsion, then the VCO can be collected. The enzymatic methods have many advantages, since the enzyme is a non-toxic ingredient, reactions accelerating agent, and active at low concentrations. The enzyme reaction speed is regulated by temperature, pH and enzyme concentration [15]. The reaction is also easily cut when the results has been desired. Another important thing using enzymes in the VOC production is enzymes categorized as a natural ingredients that is environmentally friendly [16]. One of the protease enzymes can be obtained from pineapple which according to the literature contains bromelin enzyme [17]. Based
on the background mentioned, this study was conducted to determine the effectiveness of the enzymes contained in the fresh pineapple fruit juice in the production of VCO.

2. Method

2.1. Chemicals

The main ingredient is coconut and raw pineapple which were obtained from Purworejo, Central Java. Chemicals such as NaOH, KOH, oxalic acid, alcohol, HCl, phenolphthalein, and distillated water were obtained from Brataco.

2.2. VCO production

One kg of grated coconut was added with 3 liters of clean water and the coconut milk is taken, then let it settle for 6 hours until a layer of cream and water are formed. The top layer of cream was taken and measured using measuring cylinder for 100 mL then put into countainer. While preparing the coconut cream, the pineapple juice was prepared by mincing 500 grams of raw pineapple using blender then filtered. Pineapple juice was then put into each container containing coconut cream at different concentrations of 0 mL (control), 5 mL, 10 mL, 15 mL, 20 mL, 25 mL, and 30 mL, respectively (Table 1). After that, the cream and pineapple juice were homogenized and let sit for 24 hours. In the end of 24 hours, it formed 3 layers, namely "blondo", oil and water. The oil layer is in the middle layer. Separation of the oil was done by drawing one by one from the lowest layer. The volume of the oil the measured to study the effect of enzyme concentration in the VCO production. All the experiments were done in 3 times. Water content, free acid number, and saponification number were tested to analyze the quality of VCO. The interpretation of the measuring data was according to Codex Stand.

| Group | Coconut milk (mL) | Raw pineapple juice (mL) |
|-------|------------------|-------------------------|
| 1     | 100              | 0                       |
| 2     | 100              | 5                       |
| 3     | 100              | 10                      |
| 4     | 100              | 15                      |
| 5     | 100              | 20                      |
| 6     | 100              | 25                      |
| 7     | 100              | 30                      |

3. Results and Discussion

In this study, the VCO has been produced by enzymatic method. The enzyme was obtained from fresh raw pineapple juice. Pineapple juice (Ananas comosus) contains a protease called bromelin [18]. Bromelin is able to hydrolyze peptide bonds in proteins or polypeptides into amino acid molecules [19]. Addition of solutions containing proteases into coconut milk cream will break down the proteins that act as emulsifiers in coconut milk. Thus, the VCO is able to be extracted from coconut milk cream [20]. Enzymatic hydrolysis of peptide is depicted in Figure 1.
In the study of the effect of enzyme concentration, seven different experiments were done. All the experiments were conducted for 3 times to obtain the correct data. The experiments were started by preparing coconut milk than separation of coconut cream from the milk by settling the milk for 6 hours. Seven (7) clean containers were then filled with 100 mL of coconut cream and raw pineapple juice at 7 different volumes. The variation of the experiments and the VOC produced at 7 different experiments are explained in Table 2.

Table 2. VCO production in different concentration of raw pineapple juice

| Group | Coconut cream (mL) | Raw pineapple juice (mL) | Volume of VCO |
|-------|--------------------|-------------------------|---------------|
|       |                    |                         | Data 1 | Data 2 | Data 3 | Mean ± SD (%) |
| 1     | 100                | 0                       | 17.00  | 16.90  | 17.20  | 17.03 ± 0.15  |
| 2     | 100                | 5                       | 17.50  | 17.50  | 17.60  | 17.53 ± 0.06  |
| 3     | 100                | 10                      | 20.00  | 19.80  | 21.40  | 20.40 ± 0.87  |
| 4     | 100                | 15                      | 22.50  | 22.30  | 22.50  | 22.43 ± 0.12  |
| 5     | 100                | 20                      | 24.00  | 23.90  | 23.70  | 23.86 ± 0.15  |
| 6     | 100                | 25                      | 24.20  | 24.10  | 23.90  | 24.06 ± 0.15  |
| 7     | 100                | 30                      | 24.30  | 24.20  | 24.30  | 24.26 ± 0.06  |

Based on Table 2, in the 0 mL of raw pineapple juice added produce 17.03 ±0.15 mL of VCO. This may due to the production of VCO conducted by enzyme that come from the bacteria are available in the system. This can happen since the process was done in non-aseptic work and also was done without any sterilization process. In the increase of raw pineapple juice added, the VCO that is produced is increasing until in the certain level. It can be seen in the Figure 1, the VCO produced reach around 24 mL when 20 mL of raw pineapple juice was added. The higher concentration of pineapple juice added does not increase the VCO. A clear explanation can be seen also in Table 3. The production rate of VCO does not increase in the increase of raw pineapple juice added. This may due to all the substrate (coconut milk) have been reacted with the enzymes [22].

![Figure 1. Enzymatic hydrolysis of peptide [21].](image-url)
Figure 1. Profile of VCO production in the difference concentration of pineapple juice

Table 3. Production rate of VCO in different concentration of raw pineapple juice.

| Group | Raw pineapple juice (mL) | VCO produced (mL) | Fermentation period (minute) | Production rate (mL minute$^{-1}$) |
|-------|--------------------------|-------------------|-----------------------------|-----------------------------------|
| 1     | 0                        | 17.03 ± 0.15      | 1440                        | (1.10 ± 0.010) x 10$^{-2}$        |
| 2     | 5                        | 17.53 ± 0.06      | 1440                        | (1.20 ± 0.004) x 10$^{-2}$        |
| 3     | 10                       | 20.40 ± 0.87      | 1440                        | (1.40 ± 0.060) x 10$^{-2}$        |
| 4     | 15                       | 22.43 ± 0.12      | 1440                        | (1.50 ± 0.008) x 10$^{-2}$        |
| 5     | 20                       | 23.86 ± 0.15      | 1440                        | (1.60 ± 0.010) x 10$^{-2}$        |
| 6     | 25                       | 24.06 ± 0.15      | 1440                        | (1.60 ± 0.010) x 10$^{-2}$        |
| 7     | 30                       | 24.26 ± 0.06      | 1440                        | (1.60 ± 0.004) x 10$^{-2}$        |

The quality of VCO was evaluated by measuring water content, free acid number, and saponification number. While, the interpretation of the quality was according to Codex Stan. The tabulation of quality evaluation of VCO can be seen in Table 4.

Table 4. Quality evaluation of VCO.

| Evaluation | Water content (%) | Free fatty acid (%) | Saponification number (mg KOH) |
|------------|-------------------|---------------------|--------------------------------|
| 1          | 0.34              | 0.26                | 264.77                         |
| 2          | 0.35              | 0.30                | 264.22                         |
| 3          | 0.36              | 0.31                | 262.25                         |
| Mean ±SD   | 0.34 ±0.01        | 0.29±0.03           | 263.75±1.33                    |
| Standard (Codex Stan) | 0.10 – 0.50 | Less than 0.5 | 250-260 |
The water content is interpreting the amount of water contained in the VCO [23]. The measurement of water content was done by weighing as much as 5 grams of VCO in the evaporating disc and then placed into 105 °C oven until constant weight. The calculation of water content was based on Equation 1.

\[
Water \ content = \frac{A-B}{A} \times 100\%
\]  

(1)

Where A is 5 grams of VCO and B is weight of dried VCO in the 105 °C oven until constant weight. In the 3 times measurement, the water content was found to be 0.34 ± 0.01 %. According to Codex Standard, the water content of VCO produced met to the standard, which is between 0.1-0.5% [24]. This water content is necessary to be evaluated since the presence of water in the VCO will initiate the hydrolysis reaction to produce fatty acid and glycerol. The hydrolysis reaction undergoes to rancidity process of VCO [21, 26, 27].

The number of free fatty acid measurement was done for 3 times measurement (Table 4). The average number of free fatty acid of VCO produced was found to be 0.29 ± 0.03 %. According to the Codex Stan, the number of free fatty acid of VCO produced met the standard, i.e. did not exceed than 0.5% [24]. There is a very close correlation between the number of free fatty acids and water content. According to Frega (1999), the presence of free fatty acids is the result of hydrolysis process of oil or fat. The more water contained in the oil leads the more free fatty acids found in the oil [26, 27].

The saponification number of VCO produced was found to be 263.75 ±1.33 mg KOH. This data is slightly higher than the Codex Stan, which mentioned 250-260 mg NaOH/KOH [24]. The larger saponification numbers of VCO produced may due to the VCO which contains high lauric acid i.e. a medium carbon chain (MCFA) in the large amount. The medium carbon chain takes the responsibility of the higher saponification number [21].

4. Conclusion

Based on the experimental data, the production rate of VCO increase in the presence of enzyme which is available in the raw pineapple juice. The quality of VCO produced meet the standard available.

5. Acknowledgment

The facility and financial support from Universitas Muhammadiyah Yogyakarta to the authors are highly acknowledged.

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