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The Potential of Crude Extract Bromelain Enzyme on Production of Virgin Candlenut Oil (VCdO)

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Abstract

Virgin Candlenut Oil (VCdO) is carried out enzymatically without using high-temperature heating and does not use organic solvents so it produces an oil with good quality and low FFA levels. Bromelain enzyme is sourced from pineapple hump. This study aims to determine the optimal conditions of the characteristics of VCdO including yield and free fatty acid (FFA) levels. Analysis of the fatty acid composition of VCdO was characterized by Gas Chromatography (GC). The manufacture of VCdO was carried out using 3 parameters, namely variations in time, temperature, and the amount of catalyst (enzyme). Optimization of the yield and FFA levels was determined by the response surface method (RSM) based on the Box-Behnken experimental design with 3 factors and carried out as many as 15 experiments. The optimum yield of VCdO was 51.12% at 37 °C, 34 hours, and enzyme volume 28 mL. The lowest level of FFA VCdO was obtained at 1.213% at 37 °C, 12 hours, and enzyme volume 23 mL. The results of VCdO analysis with GC showed that the highest fatty acid content was linoleic acid at 43.73%. The FFA levels have obtained the quality standard of candlenut oil according to SNI 01 -4462 -1998.

Keywords: Virgin candlenut oil, crude extract bromelain enzyme, free fatty acid

Introduction

Candlenut oil is obtained from candlenut which is many benefits in the field of food and non-food. One of the benefits of candlenut oil is as the main ingredient for making hair oil, shampoo, and eyebrow pomade which is useful for nourishing hair (Handayani et al., 2020; Ulfah & Sulandjari, 2018).

The candlenut oil extraction process can be carried out using several methods, namely, soxhletation, pressing, and so on. The soxhletation method is carried out using organic solvents (non-polar solvents) and using high-temperature heating (Arlene, 2013; Yamlean et al ., 2019). The pressing method is carried out by a cooking or roasting process at a temperature of around 115.5 °C and pressed using a mechanical pressing method (Sutiofani et al., 2021). The extraction method using high-temperature heating brings about an oxidation process so that it can increase FFA levels which will cause a decrease in the quality of candlenut oil (Emmaputri et al., 2018; Shaah et al., 2021). The quality of the oil obtained depends on the extraction process applied.

One of extracting methods of candlenut oil without using high-temperature heating is by using the enzymatic method. Similar to VCO (Virgin Coconut Oil), the method production of VCdO (Virgin Candlenut Oil) is not carried out by heating at high temperatures and does not use organic solvents where it is feared that it will damage the structure of the compounds contained in the oil (Prayitno, 2019; Senphan & Benjakul, 2017). In addition, the enzymatic method also does not require additional expensive costs because generally pineapple (as a source of enzymes) is getting in affordable price (Fitriani et al., 2021). In terms of quality, the oil produced is with low FFA levels so the oil is not easily rancid (saved for a long time) (Rahmalia & Kusumayanti, 2021).

The enzymatic extraction of candlenut oil (VCdO) has never been carried out but for coconut oil (VCO) it has been widely used so that it can be used as a reference. The enzymatic method can be carried out on candlenut because candlenut contains protein, as well as the content in coconut (Basarang et al., 2020).

Production of VCO with the enzymatic method using a protease enzyme where the protein

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bonds of the oil in the coconut milk emulsion will be broken down with the help of the protease enzyme (Prihanani et al., 2013; Rindawati et al., 2020). One of the enzymes that can be used to break these bonds is the bromelain enzyme. Bromelain enzyme is a proteolytic enzyme found in the stem, weevil, and flesh of pineapple (Ananas comosus). Bromelain enzymes are found in young and old pineapples (Sinaga et al., 2017). The activity of the bromelain enzyme from the pineapple hump is higher than the other parts of the pineapple, so it affects the yield produced in the manufacture of VCO (Prayitno, 2019).

Based on the research reported by Soo et al. (2020) VCO can be made enzymatically using extracts from pineapple fruit and yields a high oil yield of 77.7%.

Therefore, in this study researchers are interested in researching the production of Virgin Candlenut Oil (VCdO) enzymatically by utilizing pineapple as an extract of the bromelain enzyme. The manufacture of VCdO is carried out with 3 parameters, namely variations in time, temperature, and the amount of enzyme catalyst used. To reduce the number of treatments, the Box Behnken experimental design was carried out. The use of this new method in the manufacture of virgin candlenut oil is expected to provide novelty to improve the processing method of candlenut oil with better quality.

Methods

The tools used in this study were glassware (*pyrex*), centrifuge (Eppendorf 5804), centrifuge tube, shaker incubator, analytical balance (*Ohaus*), knife, buret (*pyrex*), hot plate, Gas Chromatography (GC) instrument Shimazu QP 2010.

The materials used in this study were candlenut, pineapple, ethanol (*Merck*, 96%), NaOH (*Merck*, 0.1 N), phenolphthalein, and *aquadest*.

Preparation of candlenut cream

Candlenut mashed using a blender with water added in a ratio (1:1). Then squeezed to produce candlenut cream. After that, candlenut cream is put into a beaker glass and allowed to

stand for 24 hours until it separates into two layers, namely the top layer in the form of cream (the thick part) and the bottom layer in the form of skim (watery). Next, the cream part that will be used for the manufacture of VCdO is taken.

Preparation of crude extract bromelain enzyme from pineapple hump

Peel the skin of the pineapple with a knife. Washed with clean water. Then separate the pineapple flesh from the hump. Then the pineapple hump is taken and washed until clean. Then cut into small pieces and put into a blender to be mashed. Then filtered to obtain pineapple hump extract. The extract obtained was further tested for the identification of the bromelain enzyme.

VCdO production

100 grams of candlenut cream was put into a glass beaker. Then, added 35 mL of crude extract bromelain enzyme was. Stirred. Then the glass beaker was covered with aluminum foil and incubated at 43 °C for 12 hours. After incubation, 3 layers will be formed, the top layer is blondo, the middle layer is oil, and the bottom layer is water. To separate the oil from the blondo, centrifugation was carried out for 15 minutes at a speed of 3000 rpm. The oil yield was calculated using the following equation (1):

$$Yield = \frac{weight\ of\ extracted\ oil}{weight\ of\ cream}\ x\ 100\% \qquad (1)$$

The same procedure was carried out for variations in temperature, incubation time, and volume of crude extract bromelain enzyme using the Box Behnken experimental design.

Table 1. Box Behnken experimental design of production VCdO

No.	Temperature (°C)	Time (hour)	Enzyme Volume (mL)
1.	43	12	35
2.	43	24	25
3.	43	24	25
4.	43	12	15
5.	49	12	25
6.	49	24	35
7.	37	24	35
8.	37	24	15
9.	49	24	15
10.	49	36	25
11.	37	12	25
12.	43	24	25
13.	43	36	15
14.	37	36	25
15.	43	36	35

Response Surface Method (RSM) with Box Behnken experimental design was used to obtain optimum conditions using SigmaXL software version 8.

Determination of free fatty acid (FFA)

Weighed 4 g of VCdO into a 250 mL Erlenmeyer, then added 50 mL of 96% ethanol. After that, added 3 drops of phenolphthalein indicator and titrated with 0.1 N NaOH standard until the pink color remained (unchanged for 15 seconds). The free fatty acid content was calculated using the following equation.

$$\%FFA = \frac{M\ x\ V\ x\ T}{10\ m} \qquad (2)$$

Where M is the molecular weight of fatty acids as linoleic acid (g/mol), V is the volume of titrant (mL), T is the normality of NaOH (N), and m is the weight of the sample (g).

Results and Discussion

Results of preparation and identification of bromelain enzyme

Crude bromelain enzyme was extracted from mashed pineapple hump. From 3 pineapples, 407.28 grams of pineapple hump were obtained and 356 mL of pineapple hump extract (crude extract bromelain enzyme) was obtained, the extract obtained was fresh yellow.

Crude bromelain enzyme extract was identified using the PbS. This test was conducted to determine the presence of elemental sulfur in the amino acid cysteine. The test procedure was carried out by adding 10% NaOH solution into 2 mL of pineapple hump extract solution (crude extract bromelain enzyme) and then heating for 5 minutes. The addition of NaOH aims to denature the sulfur protein contained in the amino acid cysteine to break down into sulfide ions. Furthermore, with the addition of Pb-Acetate, sulfide ions released from cysteine will react with Pb2+ ions from Pb-Acetate to form a black precipitate, PbS (Silaban et al., 2014).

The results showed the formation of black PbS salt (in the form of precipitate) in the crude extract bromelain enzyme. This is in line with Prayitno, (2019) who identification in pineapple extract indicate through discoloring and found the sediment solution which showed a positive presence of bromelain enzyme.

VCdO production

VCdO will be formed when the cream emulsion system is damaged. Protein which is an emulsifier in candlenut cream will be degraded through a hydrolysis process with the help of the bromelain enzyme which is a hydrolase enzyme on protein substrates.

Protein breakdown causes the emulsion system to become too unstable so that it can be separated into three layers, namely blondo, oil, and water layers. Due to the difference in specific gravity, the blondo layer is at the top, the oil layer is in the middle, and the water layer is at the bottom.

The surface response method with the Box-Behnken experimental design was used to determine the optimum conditions for VCdO yield. The prediction of the amount of VCdO yield formed is shown in T**able 2**.

Table 2. Yield and prediction of VCdO yield

No.	Temperature (°C)	Times (Hour)	Extract Bromelain Enzyme Volume (mL)	VCdO Yield (%)	Predicted (Fitted) Value %
1.	43	12	35	24.1	32.700
2.	43	24	25	28.5	38.167
3.	43	24	25	29.2	29.938
4.	43	12	15	24.1	14.763
5.	49	12	25	22.8	22.725
6.	49	24	35	21.9	21.975
7.	37	24	35	43.4	38.167
8.	37	24	15	42.6	38.167
9.	49	24	15	19.9	29.313
10.	49	36	25	14.3	23.638
11.	37	12	25	58.8	50.200
12.	43	24	25	30.1	20.688
13.	43	36	15	23.8	22.988
14.	37	36	25	40.3	39.563
15.	43	36	35	14.3	15.113

The yield of VCdO using the response surface from the influence of temperature and incubation time can be seen in **Figure 1 (A).** The highest VCdO yield was in the range of 50-55% with the temperature at 37 °C and an incubation time of 33.6 hours.

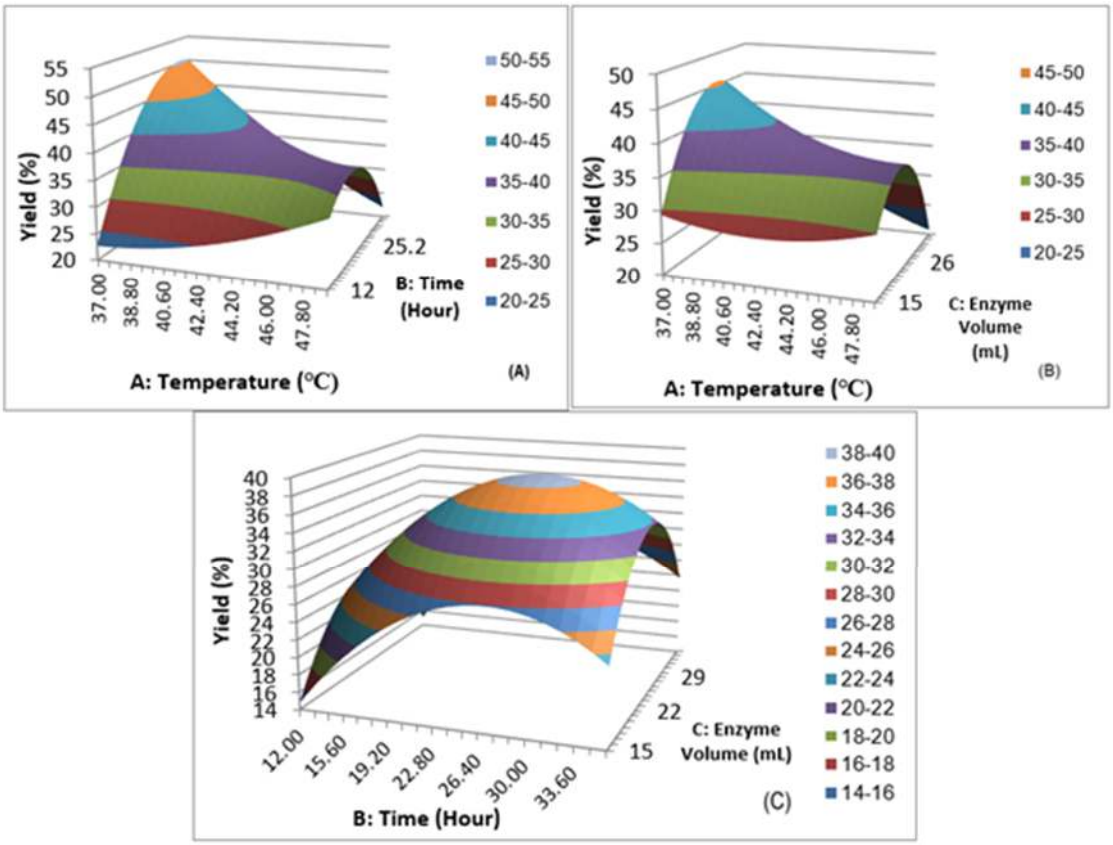

Figure 1. RSM of VCdO yield from effect of (A) temperature and incubation time; (B) incubation temperature and enzyme volume; (C) incubation time and enzyme volume

Based on the graph in **Figure 1 (B)** shows that the highest VCdO yield is in the range of 45-50% at 37 °C and 27 mL enzyme volume. Based on the graph in Figure 1 (C) shows that the highest VCdO yield is in the range of 38-40 % obtained at an incubation time of 26.4 hours and 25 mL enzyme volume.

Based on the RSM graph shown in **Figure 1 (A); (B) and (C)**, which were tested statistically, the optimum predictive conditions for obtaining the highest levels of VCdO using crude extract bromelain enzyme extract were at 37 °C, 34 hours and the volume of crude bromelain enzyme extract was 28 mL, where the VCdO yield was 51.12%.

It can be seen from the picture that the more bromelain enzyme extract added, the higher the VCdO level, this is following (Prihanani et al., 2013) and (Suirta et al., 2021) that the more enzymes added to the manufacturing process VCO will increase the oil yield.

Generally, each enzyme has a maximum activity at a certain temperature, the enzyme activity will increase with increasing temperature until the optimum temperature is reached. In addition, a further increase in temperature will cause a decrease in enzyme activity. In VCdO bromelain enzyme extract, the optimum level was at 37 °C. This is under Harimurti et al. (2022) that the production of VCO with variations in incubation temperatures of 30 °C, 50 °C and 80 °C obtained the highest yield at the lowest temperature, it is in 30 °C. Then the levels decreased with increasing temperature. The increase in the rate of enzyme activity below the optimum temperature is caused by an increase in the kinetic energy of the reacting molecules which results in the loss of the biological activity of the enzyme (Rifdah et al., 2021; Soo et al., 2020).

Free fatty acid (FFA) levels

The surface response method with the Box-Behnken experimental design was used to determine the optimum conditions for ALB VCdO levels. The prediction of FFA VCdO formed is shown in T**able 3**.

Table 3. FFA and prediction of FFA VCdO

No.	Temperature (°C)	Times (hour)	Extract Bromelain Enzyme Volume (mL)	FFA (%)	Predicted (Fitted) Value %
1.	43	12	35	1.63	1.879
2.	43	24	25	1.62	1.600
3.	43	24	25	1.60	1.920
4.	43	12	15	1.58	1.551
5.	49	12	25	1.87	1.216
6.	49	24	35	2.07	2.084
7.	37	24	35	1.35	1.600
8.	37	24	15	1.26	1.600
9.	49	24	15	1.90	1.273
10.	49	36	25	2.10	1.829
11.	37	12	25	1.20	1.371
12.	43	24	25	1.58	2.058
13.	43	36	15	1.72	1.716
14.	37	36	25	1.38	1.330
15.	43	36	35	1.80	1.634

FFA VCdO levels using the response surface from the influence of temperature and incubation time can be seen in **Figure 2 (A)**. The lowest FFA VCdO was in the range of 1.2-1.4 % with a temperature of 37 °C and an incubation time of 12 hours.

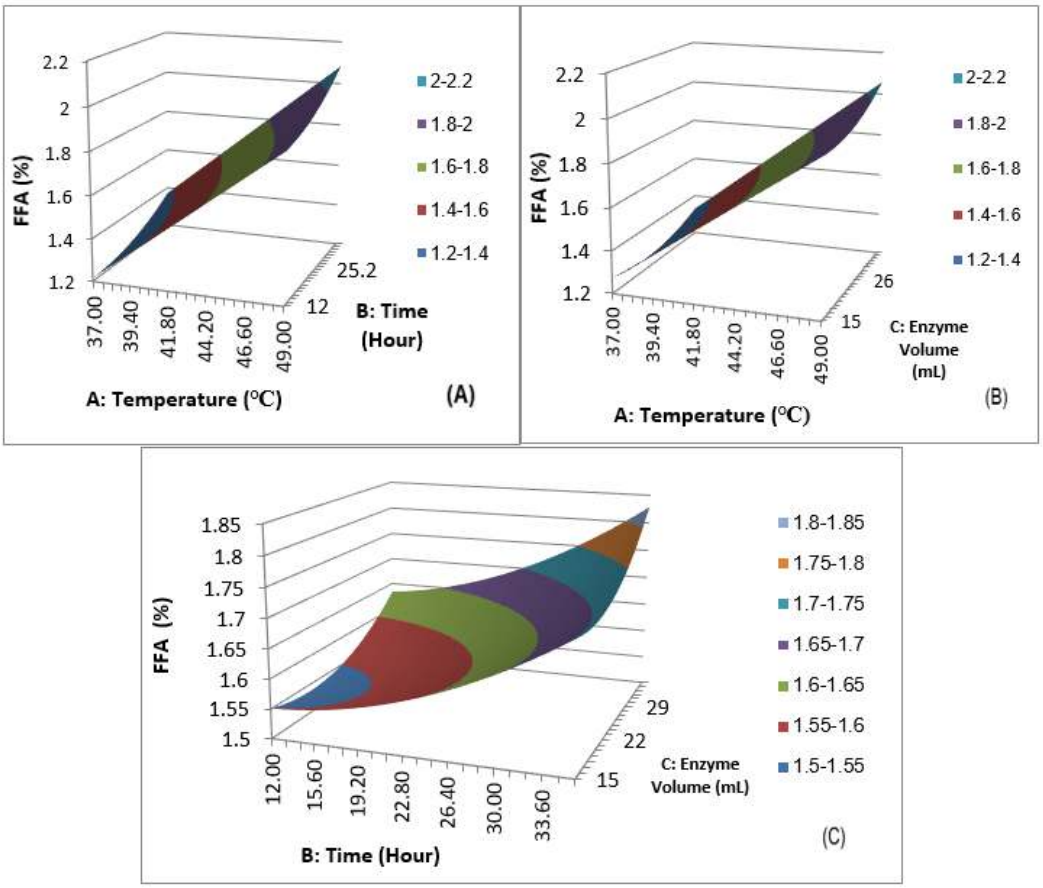

Figure 2. RSM of FFA VCdO from effect of (A) temperature and incubation time; (B) Incubation temperature and enzyme volume; (C) incubation time and enzyme volume

Based on the graph in **Figure 2 (B)** shows that the lowest FFA is in the range of 1.2-1.4% with the temperature at 37 °C and the volume of the enzyme is 22 mL. Based on the graph in **Figure 2 (C)** shows that the lowest FFA is in the range of 1.5 – 1.55% which is obtained with 20 mL enzyme volume for 12 hours.

Based on the RSM shown in **Figure 2 (A); (B) and (C),** which were tested statistically, the optimum predictive conditions for obtaining the lowest FFA in VCdO of bromelain enzyme extract were at 37 °C, 12 hours, and the volume of bromelain enzyme was 23 mL, where FFA was obtained in 1.213 %.

This has obtained the quality standard of candlenut oil according to SNI, where FFA levels according to SNI are in the range of 0.10-0.15%. For FFA levels, the optimum value is seen at the lowest FFA levels. This is because the more oil with good quality, the lower the FFA levels.

FFA is one of the most important oil quality parameters. Oils with high FFA will not be saved for a long time (easy to go rancid). FFA levels are affected by temperature, the higher the temperature, the greater the possibility of an oxidation process which can increase the FFA levels (Rahmalia & Kusumayanti, 2021).

Based on research reported by (Roni et al., 2022) that the length of incubation time affects FFA VCO. The longer incubation time and a large amount of pineapple hump extract used resulted in accelerated hydrolysis due to the water content in the VCO pineapple hump which could increase FFA levels. This is following the results of the study that the incubation period to get the lowest FFA levels at 12 hours.

Analysis of VCdO fatty acid composition with gas chromatography (GC)

Analysis of the fatty acid composition of VCdO was characterized by using Gas Chromatography (GC). The results of the characterization of the fatty acid composition of VCdO are shown in **Figure 3**.

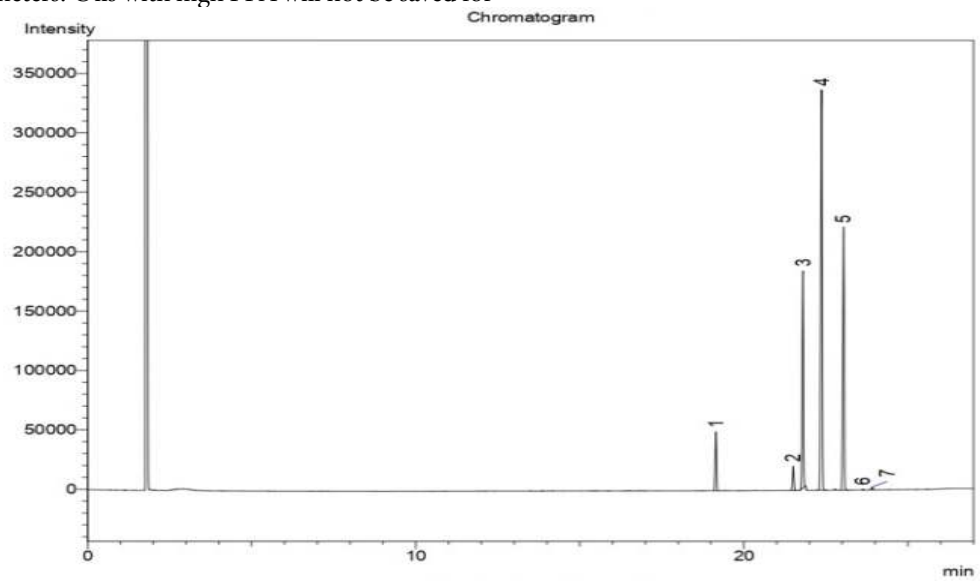

Figure 3. Chromatogram fatty acid composition of VCdO

Figure 3 shows that there are 7 types of fatty acids identified in VCdO which are shown in **Table 4**.

Based on the data from **Table 4**, the results of the analysis of fatty acids in VCdO (Virgin Candlenut Oil) using Gas Chromatography (GC) obtained 7 types of fatty acids consisting of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), acid linolenic acid (C18:3), eicosanoid acid (C20:0). and 9-eicosanoic acid (C20:1).

Table 4. Composition of fatty acids contained in VCdO

Fatty Acid	Percentage (%)
C16:0 (Palmitic Acid)	5.58
C18:0 (Stearic Acid)	2.48
C18:1 (Oleic Acid)	21.60
C18:2 (Linoleic Acid)	43.73
C18:3 (Linolenic Acid)	26.22
C20:0 (Eukosanoic Acid)	0.07
C20:1 (9-eikosanoic Acid)	0.32
	100

The results of the Gas Chromatography (GC) test showed that the fattiest acid content is linoleic acid at 43.73%. This is per the research reported by Pham et al. (2018) that the highest fatty acid content produced from candlenut oil is linoleic acid.

Conclusions

Virgin Candlenut Oil (VCdO) has been through enzymatically using crude extract bromelain enzyme. The optimum condition of VCdO characteristics was determined by Response Surface Methodology (RSM) based on the Box-Behnken experimental design. The optimum condition of VCdO yield of 51.12% was obtained at 37 °C, 34 hours and the enzyme volume was 28 mL. The lowest FFA VCdO levels in 1.213% were obtained at 37 °C, 12 hours, and 23 mL of enzyme volume. The optimum results of FFA VCdO levels have obtained the quality standard of candlenut oil according to SNI. The results of VCdO analysis with Gas Chromatography showed that the highest fatty acid content was linoleic acid at 43.73%.

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