Vol. 12, No. 4 (2023) : 988 - 996
DOI : http://dx.doi.org/10.23960/jtep-l.v12i4.988-996
JURNAL TEKNIK PERTANIAN LAMPUNG
ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

The Effect of Yeast Dose and Fermentation Time on the Quality of Virgin Coconut Oil (VCO)

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Article History :
Received : 4 April 2023
Received in revised form : 7 July 2023
Accepted : 8 July 2023

Keywords :
Virgin coconut oil,
Yeast,
Time,
Yield,
Free fatty acid

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ABSTRACT

Coconut are tropical plants and can be easily found in Indonesia. Virgin Coconut Oil (VCO) is one of the products produced from coconut beans. VCO is processed using fermentation techniques to maintain its quality and purity. This study aimed to determine the effect of yeast dosage and fermentation time on VCO yield, water content, and free fatty acids. Yeast dosage and fermentation time were the two treatment factors used in this study with a factorial Completely Randomized Design (CRD). To assess the impact of each treatment, the data was analyzed by using ANOVA with the significance level of 95%, followed using the Zeleny method analysis to find the best treatment condition. The oil found in this study was in compliance with SNI 7381:2008 and Asian Pacific Coconut Community (APCC) standards. The effect of yeast dose and fermentation period on oil yield, water content, and free fatty acid content in VCO was significant at α = 0.05. The results of the analysis showed that the best treatment condition was found at a yeast rate of 10 g and a fermentation time of 20 h. This treatment resulted about 15.67% oil yield with 0.2% moisture content and 0.2% free fatty acids.

1. INTRODUCTION

As a tropical country, Indonesia consists of thousands of islands, and is known as the largest coconut producer in the world (James, 2021). Based on data from the Directorate General of Plantation (Ditjenbun, 2021), almost all provinces in Indonesia have community coconut gardens which can be used to develop various useful processed coconut products. The existence of coconut plants is very important in human life because all components of coconut can be utilized optimally.

One of the processed products from the coconut plant that is most popular and has high economic value is virgin coconut oil (VCO) (Arief *et al.*, 2022). There are many studies that examine coconut oil, especially in relation to VCO or pure coconut oil which is produced without heating or with low heating (Aditiya *et al.*, 2014). Wiyani *et al.* (2020) revealed that VCO has the potential to improve human health, so it can be used in

cosmetics and medicinal ingredients (Amit *et al.*, 2022).

Data from the Central Statistics Agency (BPS, 2021) shows that the total VCO imported into Indonesia reached an average of 387 tons/year from 2015 to 2020, while the amount of VCO produced in Indonesia was only around 10,851 tons/year, and the need for VCO in Indonesia reached 175,360 tons. /year. This BPS data shows that Indonesia is still short of VCO supplies. This fact was also confirmed by the North Central Timor Industry and Trade Service (Disperindag Timor Tengah Utara, 2022), which reported that VCO production in North Central Timor Regency is still low, only carried out by two groups of craftsmen in Aplasi Village (Kefamenanu City District) and Taekas Village (East Miomafo District). These two groups still produce VCO at low and unsustainable capacities, and production depends on consumer demand. The high demand for VCO is due to its content which is very beneficial for health.

Based on research by Rachmawati *et al*. (2022), around 90% of the fatty acids in VCO are saturated and the remaining 10% are unsaturated fatty acids. The most saturated fatty acids in VCO are lauric acid, around 53% and caprylic acid, 7%. The high concentration of saturated fatty acids in VCO can prevent rancidity due to oxidation. According to Alvionita *et al.* (2016), the caprylic, capric and lauric acid content in VCO is known to have a special function, because VCO can be used as an effective antifungal, antibacterial, antiprotozoal and antiviral for the body (Pulung *et al*., 2016). Around 92% of the VCO content includes medium and short chain fatty acids, which the body can use to produce energy (Safitri *et al*., 2022). Saturated fatty acids in VCO do not have double bonds like unsaturated fatty acids so they are more resistant to exposure to light, heat and oxidation so they can be stored for a long time (Aziz *et al*., 2017).

The right processing process will produce high quality VCO. In general, the processing of coconut oil made by the community is conventional. However, conventional use of high temperature heating can change the structure of the oil and create an oil color that is cloudy and easily rancid (Andaka & Arumsari, 2016). Making VCO using relatively low temperatures is a solution to reduce these losses.

Ndife *et al*. (2019) stated that several methods can be used to produce VCO, namely fermentation, centrifugation, freezing and solvents. In the fermentation method, yeast and fermentation time are factors that greatly influence the quality of VCO. Yeast is used as a starter to break down the emulsion of cream or coconut milk to produce VCO using the fermentation method (Maharun & Apriyantono, 2014). The research results of Fathurahmi *et al.* (2020) revealed that the treatment of various levels of bread yeast and fermentation time had no significant effect on the yield, but had a significant effect on the water content and free fatty acids of VCO. Optimal fermentation time can produce high yields with optimal water content and low free fatty acids so that the taste and aroma of VCO are maintained. According to Tari *et al*. (2021), a fermentation time that is too short can make the quality of the VCO poor, while a fermentation time that is too long can cause the VCO to become too sour and smell rancid. Therefore, the aim of this research is to determine the effect of Saccharomyces cerevisiae yeast dosage and fermentation time on the yield and quality of VCO.

2. MATERIALS AND METHODS

The research was carried out from December 2022 to February 2023 at the Laboratory of the Agriculture Faculty and the Chemistry Laboratory at the University of Timor. The process of making VCO requires materials which include fresh old brown coconut flesh obtained from coconut plantations in Raimataus Village, white sugar and brown sugar

obtained from the local market, baker's yeast with the brand Fermipan, and coconut water. Meanwhile, the chemicals used for analysis included 95% alcohol containing 95% ethyl alcohol, phenolphthalein (PP) indicator, NaOH solution with a concentration of 0.05 N, and distilled water.

The equipment used to make VCO involved a basin, machete, knife, coconut milk filter, small clear hose, scales, label paper, jar, thermometer and coarse sheet filter paper. Tools for analysis were burettes, glass funnels, statives and clamps, beakers, measuring cups, Erlenmeyer, dropper pipettes, analytical scales, and Memert ovens.

2.1. Preparation of Coconut Milk Cream

The first step in making coconut milk cream was to prepare the flesh of 70 fresh, mature coconut beans that have been cleaned of the skin and then grated them using a coconut grater machine. The 31 kg of grated coconut obtained was extracted traditionally by adding warm water to the grated coconut at a 1:1 ratio, then squeezing with a clean cloth to take the coconut milk. After that, the coconut milk was filtered and allowed to settle for one hour until two layers are formed, namely coconut cream on the top, and liquid coconut milk in the bottom. The coconut milk cream produced was 21,600 mL.

2.2. Starter Preparation

The starter was prepared by referring to the method developed by Setyorini & Lusiani (2022) where coconut water was mixed with brown sugar and white sugar in a ratio of 10:1 (2,700 mL coconut water: 270 g brown sugar and 270 g white sugar), then heated for 10 minutes until the sugar dissolves. After that, the solution was left for 20 min until it was warm, then added baker's yeast (*Saccharomyces cerevisiae*) into 100 mL of this mixture with a variation of 6 g, 8 g, and 10 g as a fermentation inoculum. The mixture was homogenized by stirring.

2.3. Making VCO

In this study, researchers used the fermentation method as described by (Setyorini & Evi, 2022). To make VCO, the top layer coconut milk cream was separated from water, then mixed with starter at a ratio of 8:1 (800 mL coconut milk cream and 100 mL starter) based on the inoculum variation studied. Next, each container was closed and fermented at room temperature for different times according to the treatment variations studied, namely 20 h, 25 h, and 30 h. After fermentation was complete, three layers were visible, namely oil (top), dreg or “blondo” (middle), and water (bottom). Using a spoon, the oil was collected. Next, the oil was filtered using filter paper to prevent mixing of water and oil.

2.4. Observed Parameters and Data analysis

The parameters included yield (Rindawati, 2020), water content using the oven method (BSN, 2008), and free fatty acid content analysis following the method carried out by Untari *et al*. (2020).

To determine the effect of each treatment, the resulting data was analyzed using analysis of variance (ANOVA) with SPSS Version 20 at a 95% confidence interval. Meanwhile, to get the best treatment the Zeleny method was used.

3. RESULTS AND DISCUSSION

3.1. Oil Yield

The effect of the combination of yeast dosage and fermentation time on VCO yield is presented in Figure 1. The research results show that VCO production tends to increase with increasing yeast dosage and fermentation time. The breakdown of proteins in coconut milk emulsions is influenced by the protease enzyme present in baker's yeast (*Saccharomyces sereviceae*) which causes the emulsion system in coconut milk to become unstable so that the oil can separate (Fitriani *et al.*, 2021). Asmoro *et al.* (2018), stated that the longer the fermentation process takes, the more oil components that can be released from the emulsion, namely water, dregs (locally called “blondo”), and oil. Therefore, there is an increase in VCO yield as fermentation time increases. This is in line with the findings of Nurida & Lusiani, (2021), where the highest VCO yield value was obtained when fermentation was carried out for 30 hours, namely 4.30% (v/v), while the lowest VCO yield was obtained when fermentation was carried out for 6 hours, namely 2.25% (v/v).

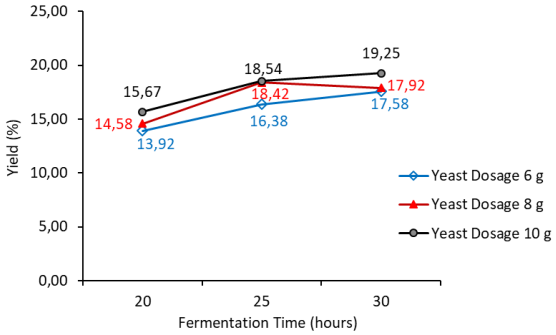

Figure 1. Effect of yeast dosage and fermentation time combinations on the VCO yield

However, the increase in yield in this study decreased at a yeast dosage of 8 g and a fermentation time of 30 hours. According to Andaka (2016), when the optimal time is reached, the active part of the enzyme is filled with substrate. As a result, the number of microorganisms that are active and capable of carrying out fermentation will decrease, so that the final yield decreases. However, the yield at a yeast dose of 10 g and a fermentation time of 30 hours did not experience a nutritional crisis, presumably because the amount of available nutrients was still sufficient to support the growth and reproduction of microorganisms during the specified fermentation time.

The results of the ANOVA analysis indicated that there was a significant difference between the effect of yeast dosage and fermentation time on VCO yield at the $\alpha = 0.05$ level. Based on Duncan's test, it was found that the yield of VCO obtained from treatment with yeast doses of 6, 8 and 10 g with a fermentation process lasting 20 hours showed a significant difference with the yield resulting from treatment with the same yeast dose and fermentation time of 25 and 30 h. This can occur allegedly because the yeast has reached its optimal growth point at an earlier time, so that increasing fermentation time will not significantly increase the yield. Therefore, the results of treatment with a yeast dosage of 10 g and a fermentation time of 30 h produced maximum yield values. The results of this study are in line with the results of a study from Fithriyatul & Evi (2021), that the highest yield value was found at a fermentation time of 30 h, namely 16.00% v/v. Meanwhile, treatment with a yeast dosage of 6 g and a fermentation time of 20 h produced the lowest VCO yield.

3.2. Water Content

The results of the research regarding the effect of yeast dosage and fermentation time on the water content of VCO are presented in Figure 2. The results of the analysis show that the water content of VCO tends to increase as more yeast is added and the longer the fermentation process takes. This research also found that the increase in water content in VCO was caused by a longer fermentation time causing the enzyme in *Sacharomyces cerevisiae* to consume all the sugar in the raw material and produce gas and water as by-products. Abudu *et al.* (2020) also added that during the fermentation process, there is a breakdown of the compounds that make up the coconut milk cream emulsion, such as carbohydrates and proteins, by yeast or lactic acid bacteria which causes the release of water from the coconut milk cream.

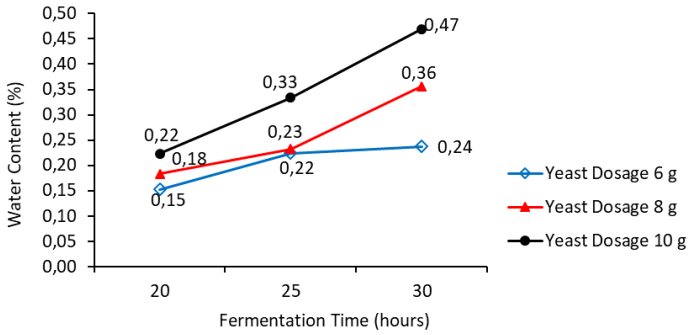

Figure 2. Effect of yeast dosage and fermentation time combinations on water content of the VCO

From the results of the analysis of variance (ANOVA), the treatment of yeast dosage and fermentation time had a significant effect on the overall VCO water content. The combination of treatment with a yeast dose of 10 g and a fermentation time of 30 hours produced the highest VCO water content with a total of 0.47%. In contrast, 6 g yeast treatment and a fermentation time of 20 hours produced the lowest water content, namely only 0.15%. Low water content in oil, according to Kusuma *et al.* (2022), can increase the shelf life of VCO. Higher water content reduces the shelf life of VCO. This also causes hydrolysis and oxidation, which increases the free fatty acid content and hydrolytic acidity of VCO (Patil & Benjakul, 2019).

According to the National Standardization Agency (BSN, 2008), the water content in VCO must not exceed 0.2% to be suitable for consumption. However, research results show that some treatments do not meet these standards. However, according to the standards set by Asian Pacific Coconut Community (APCC, 2009), VCO that is suitable for consumption has a water content that does not exceed the standard of 0.5%. Therefore, all treatments in this study resulted VCO met the APCC standard.

3.3. Free Fatty Acids

Based on the analysis results, free fatty acid (FFA) content in the VCO was in the range of 0.25% to 0.42%. Increasing the yeast dosage and fermentation time has an impact on the FFA value in VCO, as seen in Figure 3. This also correlates with the high water content, where the higher the water content in VCO, the higher the FFA value.

According to Anwar & Salima (2016), the amount of FFA in VCO increases in direct proportion to the water content. The high water content in VCO can facilitate the hydrolysis of triglycerides into free fatty acids and glycerol by the lipase enzyme found in coconut. In addition, during the extraction or storage process, the FFA content can

increase due to the reaction between oil and residual water (Mohammed *et al.*, 2021). Based on this, VCO with low water content usually has lower FFA levels, and vice versa, VCO with high water content usually has greater FFA levels. Better oil quality is indicated by low FFA (Novelena & Komari, 2022).

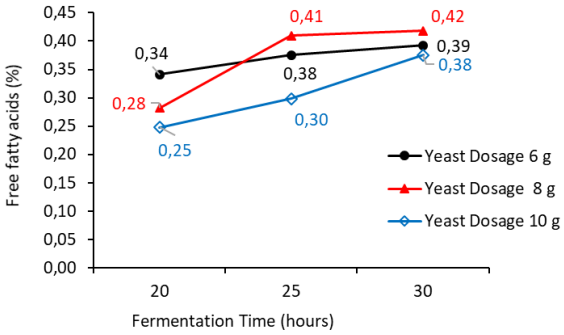

Figure 3. Effect of yeast dosage and fermentation time combinations on FFA content of the VCO

The results of the ANOVA analysis showed that there was no significant difference (α=0.05) between fermentation times of 20, 25, and 30 h with yeast dosages of 6 g and 8 g, and fermentation times of 20, 25, and 30 h with yeast dosage 10 g on the FFA content of the VCO. Treatment with a yeast dose of 8 g with a fermentation time of 30 h produced the largest FFA content in the VCO, namely 0.42%, and did not meet the SNI 7381:2008 standard which requires a maximum VCO FFA value of 0.2%. Treatment with a yeast dosage of 10 g and a fermentation time of 20 h meets SNI 7381-2008 standards because it produces the VCO with lowest FFA content, namely 0.25%. However, all yeast dosage treatments and fermentation times in this study met the requirements set by APCC (APCC, 2009), namely the maximum limit for VCO FFA of 0.5%.

3.4. Best Treatment

The selection of the best treatment is determined by comparing the ideal values for each parameter. The parameters used are yield, water content and free fatty acids. The selection of the best treatment for VCO results from a combination of yeast dosage and fermentation time using the Zeleny method (Mulyadi *et al.*, 2015). The best treatment analysis results for each treatment were obtained from VCO resulting from a yeast dosage of 10 and a fermentation time of 20 h. The parameter values for the best treatment results are presented in Table 1.

Table 1. Best treatment of yeast dosage and fermentation time to produce VCO

Test Parameter	SNI 7381-2008	APCC	Best VCO sample
Oil yield	-	-	15,67
Water content	Max 0,2%	Max 0,5%	0,22%
FFA content	Max 0,2%	Max 0,5%	0,25%

Note: from treatment combination of 10 g yeast and 20 h fermentation time.

The best treatment in this research was a yeast dose of 10 grams and a fermentation time of 20 hours resulting in a yield of 15.67%, water content of 0.22% and free fatty acids of 0.25%. These results are in accordance with research by Radhiah

et al. (2022), which states that VCO using the yeast fermentation method has a high yield and good quality. The best treatment with a yeast dosage of 10 g and a fermentation time of 20 hours meets SNI 7381-2008 standards and APCC standards which is suitable for consumption.

4. CONCLUSION

From the results of the research conducted, it was concluded that yeast dosage and fermentation time had a significant influence on the yield and quality of VCO. VCO yield increased with increasing yeast dosage and longer fermentation time. However, the quality of VCO decreased with increasing yeast dosage and longer fermentation time.

ACKNOWLEDGMENT

The author would like to thank all the supervisors, examiners and laboratory staff of the Faculty of Agriculture and the Chemistry Laboratory of the University of Timor for the permission given to us so that this research could be completed perfectly.

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