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Research article

Evaluation of yield and quality of virgin coconut oil produced using repeated batch fermentation with baker's yeast

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Abstract

Virgin coconut oil (VCO) produced using a repeated batch fermentation technique with baker's yeast was assessed for quantity and quality changes as a result of repetitive fermentation. The application of repeated batch fermentation was expected to reduce the operating costs of small industries. The batch fermentation was performed continuously six times and the yield and quality of the VCO were assessed for each batch based on one-way analysis of variance followed by the Tukey test. The results showed that the average VCO yield of the six batches was 25.71±2.09% and it was significantly reduced as repeated fermentation increased. The mean values of the moisture content and the acid and peroxide numbers were 0.01±0.007%, 0.1±0.03% and 0.1±0.05 meq/100g, respectively. The moisture and acid number were not significantly ($p > 0.05$) affected by repeated fermentation, whereas the peroxide number was significantly different. Although the peroxide number increased, it was still within the SNI 7381-2008 standards. Three cycles of repeated fermentation were recommended for the best yield of VCO.

Introduction

Virgin coconut oil (VCO) is extracted from fresh old coconuts through mechanical techniques or fermentation, without heating or using chemical substances (Villarino et al., 2007; Fife, 2005). The color of old coconuts is usually brown or gray, and they become dry as the water content decreases. Manufacturing of VCO mechanically is carried out through centrifugation of the coconut milk at approximately 12,000 rpm for 2 hr, with a lower speed and shorter time being possible but producing a lower volume (Wong and Hartina, 2014). VCO manufacture can be performed through spontaneous (using natural microbes present in coconut milk) and non-spontaneous (adding microbes to coconut milk) fermentation techniques (Jasman *et al*., 2019). The manufacturing process can be performed with or without heating at optimum temperatures for the separation of oil from water and 'blondo' in a short time (approximately 3–5 minutes), where blondo is an Indonesian term for the coconut byproduct consisting of the cream separated from the oil and water phase and made up of mainly carbohydrates, fats, proteins, and minerals (Haerani, 2010). The word "virgin" also refers to the chemical-free techniques of manufacturing coconut oils (Fife, 2005). VCO functions cover many options, including as: an energy source in food ingredients, oil for cooking and frying, a flavor enhancer and as a substitute for buttermilk in ice cream (Bawalan and Chapman, 2006). Apart from food ingredients, VCO can be used as a skin and hair conditioner, in aromatherapy, cosmetics, massage oils and skincare products (Fife, 2005; Bawalan and Chapman, 2006). In the health sector, VCO uses include strengthening the immune system, preventing atherosclerosis and heart disease, stimulating the metabolism and preventing obesity (Dumancas et al., 2016)

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Non-spontaneous fermentation of VCO can be performed using baker's yeast (*Saccharomyces cerevisiae*), 'tempeh' starter (*Rhizopus oligosporus*), and 'tape' starter (a mixture of *Aspergillus, Saccharomyces, Candida* and *Acetobacter*, where t*ape* is a kind of fermented cassava or rice in Indonesia) and these microbes are presumed to play a role through protease activity by breaking down protein bonds with oil molecules in coconut milk (Sukandar, 2011). Upon the breakdown of the polymer molecules, the oil molecules are freed, joining with one another to form their phase, separated from water and *blondo*. Although *S. cerevisiae* is better known as baker's yeast and works on carbohydrate substrates, this type of yeast has been observed to have protease activity (Younes *et al*., 2011). There are also other possible mechanisms including amylolytic microbes such as *Saccharomycopsis fibuligera* that degrade the starch components into glucose which is subsequently converted by *S. cerevisiae* into acid. The acids then lower the pH to the isoelectric levels in proteins, resulting in coagulation. Protein clumping in coconut milk breaks the emulsion, consequently separating the oil from the water and *blondo* (Suhardijono and Syamsiah, 1987).

Fermentation is defined as the process of changing a material or substrate into a product through the help of microorganisms (Hidayat et al., 2006), Industrially, it involves the intentional use of microorganisms to make products useful to humans (Paulova et al., 2013). These microorganisms usually produce enzymes that can convert raw materials (substrates) into finished products in the form of certain compounds. The conversion process is carried out by microorganism cells to obtain the compounds or elements necessary for their survival. During fermentation, the cell growth process is divided into four phases: 1) lag phase; 2) log phase; 3) stationary phase; and 4) death phase (Najafpour, 2007; Moslamy, 2019). Fermentation products are formed during microbial cell growth, and this mainly occurs during the log phase. After this phase, the substrate begins to run out and the accumulation of inhibiting compounds increases; consequently, the microbial cells begin to die. When fresh substrate is added into the fermenter before the death of the microbial cells, the remaining living cells regrow to carry out the next fermentation. Thus, such a process can be performed repeatedly and hence is known as repeated batch fermentation (Zhao et al., 2010). A pressing question is whether there are changes in the yield and quality of the product obtained from each fermentation repetition.

The VCO manufacturing business in Indonesia is mainly carried out by small-scale home industries which face the problems of small amounts of available capital and the lack of good fermentation techniques. Therefore, the current research aimed to develop a technique that addressed these problems. Based on the principle of yeast reuse, the repeated fermentation technique is expected to benefit the entrepreneurs by saving yeast and operation time while providing satisfactory yield and quality.

Materials and Methods

Repeated batch fermentation procedure

The materials used in making VCO were matured fresh coconut fruits (age approximately 12 mth) which were obtained from the Lasiana Village coconut plantation and baker's yeast (Fermipan; France) from a food store in Kupang, Indonesia.

Coconut milk preparation, starter preparation and initial batch fermentation were carried out according to Jasman et al. (2019). The coconut was peeled, cleaned and its water was removed. Then, it was grated, weighed (2 kg) and mixed with warm water (50°C) at a ratio of 1:2 (1 kg of coconut meat to 2 L of warm water). The mixture was kneaded, wrapped in a filter cloth and then squeezed to obtain coconut milk. Then, it was precipitated for 2 hr until two layers had formed, with the bottom one being water and the top one coconut cream. The water layer was removed by suction using a plastic hose.

The starter was prepared by mixing 200 mL coconut milk, 50 mL coconut water and 2 g baker's yeast in an Erlenmeyer flask. The mixture was stirred until it reached a homogeneous state; the bottle was sealed and incubated at room temperature for 12 hr.

For the initial batch procedure, 237.5 mL of coconut cream and 12.5 mL of starter were poured into a 500 mL Erlenmeyer flask and then shaken slowly to obtain a homogenous mixture. The mouth of the flask was covered using aluminum foil sealed with a rubber band and then incubated under static conditions at 30°C for 24 hr. The result consisted of three separate layers: water, oil and blondo. The oil layer was separated from the others by suctioning using a small plastic hose. The obtained oil was passed through filter paper to produce a very clear oil. The volume of oil was measured and stored in a clean glass bottle for quality analysis.

The repeated batch fermentation procedure was adapted from Ariyajarearnwong et al. (2011) and Arasaratnam et al. (2012). The yeast precipitate formed during the initial batch was separated from water using filter paper. The precipitate was put into the fermenter. The fresh coconut cream was added and stirred into a homogenous state, covered and fermented for 24 hr after which the oil, blondo and water layers were separated. The yeast precipitate found in the water layer was filtered, weighed and used for the next batch as in the previous step. This repetition was performed up to five times, totaling six batches. Due to the triplication of each batch, there were 18 fermentation units in total.

Before separating the yeast precipitate from the water phase, a 1.0 mL sample was collected from the liquid to determine the concentration of viable yeast cells. This procedure was done for each batch.

Yield calculation

The yield of the process was calculated based on the volume of VCO obtained compared to the volume of the coconut cream used based on Equation 1:

$$\text{Yield (\%)} = \frac{a}{b} \times 100 \quad (1)$$

where a is the volume of VCO and b is the volume coconut cream, both measured in milliliters.

Moisture determination

The determination of oil moisture was carried out using the thermogravimetric method according to Ketaren (2008). A sample (2 g) was weighed and placed into bottle scales. Consecutively, the bottle scales containing the oil were heated in an oven for 5 hr at 105°C, after which the oil was cooled in a desiccator and reweighed. This procedure was repeated until a constant weight was achieved. The moisture of oil was calculated based on Equation 2:

$$\text{Moisture (\%)} = \frac{B0 - B1}{B0} \times 100 \quad (2)$$

where B0 is the initial weight (before heating) and B1 is the final weight (after heating).

Acid number determination

The acid number indicates the levels of free fatty acids present in oil. High levels of free fatty acids will result in a rancid taste and odor that is unpleasant to consumers. The acid number was determined in accordance with Anonymous (1986). A sample (10 g) of coconut oil was placed in a 250 mL Erlenmeyer flask, added with 25mL of 95% alcohol and then refluxed for 1 hr. Finally, the mixture was cooled, and three drops of phenolpthalein indicator were added and then the mixture titrated with 0.1 N KOH solution until turning a pink color. The acid number was calculated based on Equation 3:

$$\text{Acid number} = \frac{\text{V KOH} \times \text{N KOH} \times \text{MW KOH}}{\text{Weight of sample (g)}} \quad (3)$$

where V KOH is the volume of KOH solution, N KOH is the normality of the KOH solution and MW KOH is the molecular weight of KOH.

Peroxide number determination

The peroxide number is the most important parameter for assessing the degree of damage to the oil (Ketaren, 2008). The level of oil oxidation can be measured by its peroxide and 2-thiobarbituric acid (TBA) numbers. The peroxide number measures the primary product of oxidation (hydroperoxide), while the TBA number measures the secondary products, which are highly diverse, making them difficult to determine simultaneously. TBA measurement focused on one secondary product (malonaldehyde acid). Thus, it is believed that the peroxide number measures all the oxidative damage to the oil, while TBA measures the off-aroma compounds (Semb, 2012).

The peroxide number was determined in accordance with Anonymous (1986) by weighing 5 g of oil in a 250 mL capped Erlenmeyer flask which was added with 30 mL of a solvent consisting of 60% acetic acid and 40% chloroform. Consecutively, the mixture was shaken until all substances dissolved and then 1 mL of KI saturated solution was added. This solution was left to homogenize for 1 min before the addition of 30 mL of distilled water followed by titration with 0.1 N $Na_2S_2O_3$ until the yellow color disappeared. Afterward, 1 mL of 1% starch indicator was added and the mixture was further titrated until the blue color disappeared. The peroxide number was expressed in milli-equivalents of peroxide in every 1,000 g of the sample based on Equation 4 and Equation 5:

$$\text{Milliequivalent/1000 g} = \frac{A \times N \times 1000}{m} \quad (4)$$

$$\text{Peroxide Number} = \frac{(V_1 - V_0) \times N \times 8}{m} \quad (5)$$

where A is the volume of sodium thiosulphate solution (in milliliters), N is the normality of sodium thiosulphate solution, m is the weight of oil (in grams), V_1 is the volume of sodium thiosulphate solution for the VCO titration (in milliliters), V_0 is the volume of sodium thiosulphate solution for blank titration (in milliliters) and 8 is the equivalence to O_2.

Data analysis

The data obtained were analyzed using a one-way analysis of variance (ANOVA) to determine the effect of repetition on the yield and quality of the VCO produced. This analysis was done using the SPSS Version 16 software for Windows (SPSS; Chicago, IL, USA). The *Tukey* test was performed to examine the significance of the data at a test level of $p < 0.05$.

Results and Discussion

Virgin coconut oil yield of repeated batch fermentation

The volume of oil produced in each batch was used to calculate the fermentation yield (Table 1) and the analysis indicated there was a significant difference among the VCO yields obtained from the six batches of repeated fermentation. Thus, it was concluded that the repetition of fermentation affected the VCO yield.

Yeast biomass of fermentation

The yeast biomass from each batch is shown in Table 2, indicating that the more repetitions, the less yeast obtained. The concentration of live yeast cells at the end of each batch (Table 2) indicates that with more repetitions of fermentation, the live cells remaining at the end of each successive batch became less and less.

Table 1 Volume and yield of virgin coconut oil obtained from repeated batch fermentation

No	Batch	Volume of oil (mL)	Coconut oil yield (% v/v)
1	Initial	25.43 ± 0.5^{a}	28.26 ± 0.6^{a}
2	Repetition 1	24.50 ± 0.5^{ab}	27.22 ± 0.6^{ab}
3	Repetition 2	24.03 ± 0.5^{ab}	26.70 ± 0.5^{ab}
4	Repetition 3	22.70 ± 1.1^{b}	25.22 ± 1.3^{b}
5	Repetition 4	21.83 ± 1.0^{bc}	24.26 ± 1.2^{bc}
6	Repetition 5	20.33 ± 0.6^{c}	22.59 ± 0.6^{c}

Mean ± SD values followed by the same lowercase superscript are not significantly ($p > 0.05$) different.

Table 2 Concentration of viable yeast cells at the end of each batch

No	Batch	Concentration of viable yeast cells (cfu/mL)
1	Initial	$(5.5 \pm 0.2) \times 10^{6a}$
2	Repetition 1	$(4.8 \pm 0.1) \times 10^{6b}$
3	Repetition 2	$(4.2 \pm 0.1) \times 10^{6c}$
4	Repetition 3	$(3.6 \pm 0.3) \times 10^{6d}$
5	Repetition 4	$(2.8 \pm 0.2) \times 10^{6e}$
6	Repetition 5	$(1.8 \pm 0.1) \times 10^{6f}$

cfu = colony forming units

Mean ± SD values followed by the same lowercase superscript are not significantly ($p > 0.05$) different.

Virgin coconut oil quality

The quality parameters of VCO examined in this study were the moisture content and the acid and peroxide numbers (Table 3). Generally, more repetitions of fermentation, the lower the quality of the oil produced. However, there was no significant difference between the reduction in quality based on the moisture content and the acid number. Furthermore, all samples still met the quality standards according to SNI 7381-2008 (Badan Standar Nasional, 2008).

The reduction in yield with repeated batch fermentation is believed to be related to the reduction in the number of *S. cerevisiae* cells that survive fermentation from the previous batch (Ariyajarearnwong et al., 2011). This assumption was supported by the data in Table 2, which shows that the more repetitions, the lower the concentration of viable yeast cells obtained. If the number of cells that survive in the previous batch is small, then the concentration of cells in the inoculum for the next batch is correspondingly small and this affects the fermentation process (Arasaratnam et al., 2012). Such decreases in living cells are related to two factors, namely the nutrient availability and the timeliness of switching from one batch to another. Regarding nutrient availability, yeast cells in this study are likely to experience a lack of nutrients because there is no supplementation before or during the fermentation process, as the nutrients used are derived solely from the coconut milk substrate. In terms of the timeliness of switching between batches, perhaps the addition of fresh substrate occurs when the yeast growth has entered the death phase; thus, the available living cells are few. This assumption was based on a study reporting that the growth of *S. cerevisiae* in a medium with an initial sugar concentration of 10% entered the stationary phase at the 20th hour (Jasman et al., 2013). Therefore, for a medium with a much lower sugar content, such as the coconut cream in the current study, both the stationary and subsequent death phases of *S. cerevisiae* cells will come earlier. Thus, if the addition of fresh cream is done after the 24th hour, the number of growable yeast cells will be less than for the previous batch. Furthermore, the Tukey test showed that the result of the initial batch was not significantly different from that of the 1st and 2nd repetitions but was significantly different from the 3rd to the 5th repetitions. Therefore, this indicated that fermentation can be carried out for three sequential repetitions without significantly reducing the yield. To achieve a high yield, it appeared that the repetition of fermentation was best limited to three times. To guarantee a high yield from the following batches, it is advisable to add supplements to the fermentation medium. Nevertheless, it is also advisable to change the substrate (switching from one batch to the next) before the death phase of yeast cell growth commences.

Table 3 Moisture content and acid and peroxide numbers of virgin coconut oil produced using repeated batch fermentation

Batch	Moisture (% w/w)	Acid number (%)	Peroxide number (meq/100g)
Initial	ND	0.067 ± 0.0^{a}	0.021 ± 0.0^{a}
Repetition 1	ND	0.067 ± 0.0^{a}	0.037 ± 0.0^{a}
Repetition 2	0.01 ± 0.01^{a}	0.080 ± 0.0^{a}	0.096 ± 0.0^{b}
Repetition 3	0.01 ± 0.01^{a}	0.106 ± 0.0^{a}	0.106 ± 0.0^{b}
Repetition 4	0.01 ± 0.01^{a}	0.126 ± 0.0^{a}	0.128 ± 0.0^{bc}
Repetition 5	0.02 ± 0.00^{a}	0.146 ± 0.0^{a}	0.154 ± 0.0^{c}
SNI 7381-2008 standard	≤ 0.5	≤ 0.2	≤ 3.0

ND = not detected

Mean ± SD values followed by the same lowercase superscript are not significantly ($p > 0.05$) different.

One-way ANOVA analysis of the moisture content (Table 3) indicated that there was no significant difference among the data from repeated fermentation indicating that this process does not affect or influence the amount of moisture in VCO. This fact suggests that the performance of *S. cerevisiae* cells in the degradation of the carbohydrate component in oil molecules remained high from the initial batch until the 5th batch. This was because if the oil granulating material in the coconut milk emulsion is not completely degraded, the resultant separation of oil and water is rendered imperfect and thus, the oil obtained would have a high moisture content (Witono et al., 2007; Jasman et al., 2019). However, the oil moisture content obtained in the current study was still far below the maximum limit according to SNI 7381-2008 (Badan Standar Nasional, 2008), meaning that the quality of the VCO produced from the initial fermentation to the 5th replication was very good in this regard.

Table 3 shows there is a slight increase in acid number starting from the initial batch to the 5th repetition. However, this increase was not statistically significant. This increase in acid number resulted from an increase in the storage period of the coconut raw material used. The storage period of the material provided a chance for the naturally occurring lipase in the material to hydrolyze the oil to free fatty acids (Lacerda et al., 2013). This was consistent with the conclusion of Ketaren (2008) that vegetable oil extracted from grains or fruits stored for a long time without undergoing the oxidation process, would have a high acid number. The batch repetitions mean that the storage period of raw materials increases, allowing hydrolysis to occur that causes an increase in free fatty acids. Although there was a slight increase in the acid number as batch repetition continued, these numbers fell below the maximum limit according to SNI 7381-2008 (Badan Standar Nasional, 2008), indicating that all the oil produced was of very good quality.

The data in Table 3 shows that the more repetitions of yeast use for fermentation, the higher the peroxide number of the coconut oil produced. There was a significant difference among the peroxide number values. Based on the Tukey test, it appeared that the initial batch and the 1st batch repetition produced almost the same value; therefore, the lowest average VCO peroxide number indicated the best oil quality. From the 2nd until the 4th batch repetition, there were similar peroxide values that were significantly higher than those of the previous batch. Thus, it was concluded that the quality of the 2nd to 4th batch repetitions was lower than for the first two results. Furthermore, the results of the 4th and 5th batch repetitions had similar peroxide values and were significantly higher than those of the previous group and thus had the lowest quality in this experiment. Hence, the increase in the peroxide number is presumed to have occurred due to the storage of the peeled coconut for a longer time. This long period of storage could have caused the enzyme lipoxidase to oxidize the oil. Although there was an increase in the peroxide number as batch repetition increased, the numbers were below the standards according to SNI 7381-2008 (Badan Standar Nasional, 2008). Consequently, the quality of VCO produced until the 5th batch repetition was very good. The increase in peroxide numbers was caused by the oxidation of unsaturated fatty acids triggered by several factors such as both ultraviolet and blue light, lipoxidase enzymes and the metal catalysts Fe and Cu (Dauthy, 1995; Ketaren, 2008). Oils with high peroxide numbers are called rancid oils, and when present in food, they are slightly bitter in taste and have a soapy scent and unpleasant odor (Belitz et al., 2009).

Therefore, based on the results and discussion above, it was concluded that the repetition of fermentation affect or reduce the VCO yield and that repeated batch fermentation did not significantly influence the quality of VCO based on the SNI 7381-2008 standard.

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