Asahan coconut for virgin coconut oil production using fermentation method

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Abstract. VCO Fermentation method can be done on a large scale with many advantages compared to the heating method, such as the effectiveness and efficiency of labour, time, cost, equipment also produce higher VCO quantity and quality. This study was aimed to determine type and yeast concentration with fermentation time that produced the best VCO yield and quality. The study was conducted from February to November 2015 in the Post Harvest Laboratory AIAT North Sumatra. The research used a completely randomized factorial design with 3 replications. The treatment consisted of 2 factors, which are yeast type (baker’s, tape, and tempeh) and yeast concentration (3, 6, 9, 12, and 15%). The results showed the baker's yeast of 15% concentration on 24 hours fermentation time gave the best yield (13.60%) and the highest score on organoleptic test. Interaction results of type and yeast concentration were significantly different on VCO water content but were not significantly different on FFA and peroxide value. Generally, the lowest VCO water content is obtained in tape yeast while the lowest FFA and peroxide value in baker’s yeast. VCO fermented by tempeh yeast produced the highest lauric acid (48.76%) while the highest medium-chain fatty acid composition produced by tape yeast fermentation (60.64%).

1. Introduction
Asahan Regency is one of the potential coconut producer regions in North Sumatra province with a total land area in 2014 of 23,342.7 hectares. Coconut production in Asahan Regency in 2014 was around 21,874 tons spread in 25 districts, where the most abundant production being in three districts, namely Silo Laut District 6,838.98 tons, Sei Kepayang 4,864 tons and Tanjung Balai 4,063 tons [1].

VCO as one of the most important coconut products besides being used in pharmaceuticals, nutraceuticals, cosmetics and industrial also used as a food ingredient in functional foods and useful for human health and nutrition contain rich medium-chain-triglycerides (MCT) [2].

VCO derived from coconuts can be extracted through dry and wet rendering. The dry rendering processed by removed and dried the coconut endosperm to create copra then crushed/pressed the copra to produce oil. The wet rendering can process by the extraction and fermentation method. VCO is still carried out conventionally by heating, so in terms of quality and quantity is still not optimal. The disadvantages of high heating in a conventional way can change the oil structure and produce the wrong oil colour. VCO processing by Fermentation be done on a large scale or household. Fermentation is carried out using microorganisms as inoculums such as bacteria and yeast. The advantages of fermentation are effectiveness in energy, relatively short time, and lower cost also does
not need complicated equipment. Some researches mentioned the advantages VCO fermentation produced more yield and clearer colour [3]; the process is easy also produce clearer oil with lower cost [4].

VCO fermentation can use several types of yeast, such as tempeh yeast, baker's yeast, and tape yeast. Rhizopus sp. in tempeh yeast produces protease enzymes that can break the lipoprotein bonds in coconut milk, thus separates the oil bonds [5]. While according to [6], bread yeast contains one type of microorganism Saccharomyces cerevisiae, which produces enzymes to convert glucose into alcohol, which acts to break the coconut milk emulsion, thus producing oil [7]. Tape yeast contains microflora such as "Shamir" to produce lipases that can break the coconut milk emulsion into oil [8].

The objectives of this study are 1. To determine the type of yeast, yeast concentration, and fermentation time that produces the highest VCO yield; 2. To determine the effect of storage time on chemical content, including water content, free fatty acid (FFA), peroxide value, and the fatty acid composition of VCO; 3. To determine the physical qualities include taste, colour, and appearance of the VCO product.

2. Methodology

2.1. Experimental materials and tools

The experiment was conducted in the Postharvest laboratory of AIAT North Sumatra from February to November 2015. The materials used in this experiment are coconuts from Asahan Regency, baker's yeast, tempeh yeast, tape yeast, and water. The tools used are knives, shredder machines, pressers, stoves, thermometers, plastic basins, modified jerry cans, hoses, buffer racks, filter paper, buckets, and pans.

2.2. Making a method of virgin coconut oil

The activity began with making a starter on coconut milk using baker's yeast, tape yeast, and tempeh yeast in several concentrations. The coconut shell is peeled, cut, and the flesh removed, the skin of the brown flesh is eroded, then shredded using shredder machine. Adding warm water (temperature 80 ° C) to the grated coconut (1.25: 1), then stirring and pressing to produce coconut milk. Coconut milk is added to the modified jerry cans and left for 2-3 hours to separate the coconut milk cream from coconut milk. Coconut milk is taken sufficiently and used to breed microbes (yeast) for one night. The concentrations of baker's yeast (R0), tape yeast (TA), and tempeh yeast (TE), which used are 3, 6, 9, 12, and 15%.

The cream is sufficiently sprinkled with coconut milk that has been fertilized by microbes from the yeast used. As a comparison (control) made VCO from the cream by directly heating the cream without the addition of microbial cultures (yeast). Then proceed with the fermentation for 6, 12, 24, and 36 hours. After successful fermentation, a layer of coconut oil will be formed at the top, blond in the middle, and water at the bottom layer. Then coconut oil is separated using 2 (two) layers of filter paper in the separating funnel.

The used sample for organoleptic, water content, free fatty acid, and peroxide value were the VCO produced by all yeasts at all concentrations with 24-hours fermentation time, while for the fatty acid analysis were fermented VCO at 15% yeast concentration with 24 hours fermentation time.

2.3. Experimental design and analysis

The research design used was a completely randomized factorial design with three replications. Data from the test results are tabulated, then analysed by analysis of variance and further tests in the form of DMRT tests at 5% level. The highest VCO yield organoleptically tested (taste, aroma, colour, and appearance). Organoleptic tests on VCO products were carried out by 10 panellists who had been trained previously. The numeric scale that used for flavours parameter are: 7 = very tasty; 6 = tasty; 5 = rather tasty; 4 = neutral; 3 = rather bad; 2 = bad; and 1 = very bad while the numeric scale for aroma, colour and appearance parameters are: 7 = really like; 6 = like; 5 = rather like; 4 = neutral; 3 = rather dislike; 2 = dislike; and 1 = very dislike.
3. Results and discussion

3.1. Virgin coconut oil (VCO) yield

VCO yield produced by fermentation using yeast Ro, Ta, and Te shows that the higher level of yeast concentration gives, the higher yield of oil produced (Table 1). The highest yeast concentration (15%) produced the highest yield of VCO in the three types of yeast (Ro, Te, and Ta) with a yield of 13.60, 13.02, and 9.06%. As for the fermentation time treatment, known that fermentation for 6 hours using tape (Ta) and tempeh yeast (Te) at all levels of concentration has not produced an oil layer.

| Concentration (%) | Fermentation time (hour) | Oil Yield (%) |
|-------------------|-------------------------|---------------|
|                   | 6                       | Ro | Ta | Te |
| 3                 | 1.20                    |    | -  | -  |
| 12                | 9.04                    | 4.25| 7.05|   |
| 24                | 11.82                   | 6.72| 10.80|  |
| 36                | 11.82                   | 6.98| 10.93|  |
| 6                 | 1.10                    |    | -  | -  |
| 12                | 9.06                    | 4.30| 7.05|   |
| 24                | 11.86                   | 7.07| 11.81|  |
| 36                | 11.91                   | 8.28| 11.69|  |
| 6                 | 1.52                    |    | -  | -  |
| 12                | 9.30                    | 4.35| 7.07|   |
| 24                | 11.35                   | 7.30| 12.1 |  |
| 36                | 11.30                   | 8.22| 12.15|   |
| 6                 | 1.50                    | -  | -  | -  |
| 12                | 9.28                    | 5.83| 7.12|   |
| 24                | 12.44                   | 7.68| 12.50|  |
| 36                | 12.46                   | 8.53| 12.57|  |
| 6                 | 1.78                    | -  | -  | -  |
| 15                | 12.90                   | 7.92| 7.23|   |
| 24                | 13.60                   | 8.57| 13.02|  |
| 36                | 13.59                   | 9.06| 12.84|  |

Fermentation time treatment for 24 hours produced the highest yield of VCO in the treatment of Ro Yeast, which was 13.60%. The 24 hours fermentation time treatment with baker’s yeast produced the highest yield of VCO that was 13.60% but generally fermentation 24 hours - 36 hours at all concentration produces high VCO yield that tends to be the same or only have a slight difference, this is due to the longer fermentation time, the results obtained was greater until the optimum point where the material has been fermented [9]. Microorganisms in yeast will ferment coconut milk by separating coconut oil in the upper layer within 24-48 hours [10].

VCO fermentation using baker’s yeast (Ro) produces a higher yield of VCO compared to 2 other types of yeast (Te and Ta). This is due to baker’s yeast (Saccharomyces cerevisiae) produce proteolytic and amyloytic enzymes where carbohydrates will change and produce acids which will lower the pH of coconut milk to reach the isoelectric point of the protein so the protein will coagulate thus facilitating the separation of oil [11]. Besides, yeast cells in baker’s yeast (Ro) have stable physiological properties, are very active in breaking down sugars and starches into carbon dioxide and alcohol, are dispersed in water, have long-lasting life, and grow rapidly [12].
3.2. Organoleptic test of virgin coconut oil
The physical quality parameters of VCO tested in organoleptic tests are taste, aroma, colour, and appearance. The criteria for aroma, taste and colour of good quality VCO as stipulated in SNI 7381: 2008 are typical scented of fresh coconut and not rancid with the typical normal taste of coconut oil and are colourless to yellowish [13] while the appearance must be clear without a mixture of ingredients/other impurities and not lumpy [14]. The results of an analysis of variance on VCO organoleptic test in Table 2 showed that the concentration and type of yeast have no significant effect on colour and appearance, but have a significant effect on the taste and aroma of VCO produced.

Generally, the highest average values of organoleptic test results for taste and aroma in three types of yeast (Ro, Ta, and Te) obtained at 15% yeast concentration. Based on organoleptic test results, baker’s yeast fermentation (Ro) has the highest average value of taste, aroma, colour, and appearance show that VCO by fermentation using baker’s yeast (Ro) is preferred by panellists compared to tempeh (Te) and tape (Ta) yeast using.

| Fermentation Treatment | Concentration (%) | Taste     | Aroma     | Colour    | Appearance |
|------------------------|-----------------|-----------|-----------|-----------|------------|
| Ro                     | 3               | 4.7 ± 1.15| 4.5 ± 1.17 | 5.3 ± 1.15| 5.2 ± 0.63 m |
|                        | 6               | 5.1 ± 1.19| 5.0 ± 0.81| 5.3 ± 1.16| 5.2 ± 0.63 m |
|                        | 9               | 5.6 ± 0.84| 5.0 ± 0.94| 5.2 ± 1.31| 5.3 ± 0.67 m |
|                        | 12              | 5.6 ± 0.84 | 5.5 ± 0.70| 5.6 ± 1.17| 5.2 ± 1.13 m |
|                        | 15              | 5.9 ± 0.73  | 5.7 ± 0.67 | 5.9 ± 0.73 | 5.7 ± 0.67 m |
| Ta                     | 3               | 3.9 ± 1.10 | 3.1 ± 0.99 | 5.0 ± 0.94 | 5.2 ± 0.42 m |
|                        | 6               | 3.8 ± 0.91 | 3.2 ± 1.03 | 5.0 ± 0.94 | 5.1 ± 0.74 m |
|                        | 9               | 3.3 ± 1.05 | 3.1 ± 1.10 | 5.3 ± 0.95 | 5.1 ± 0.74 m |
|                        | 12              | 3.2 ± 0.63  | 3.6 ± 0.69 | 5.3 ± 1.05 | 5.1 ± 0.74 m |
|                        | 15              | 3.9 ± 1.19 | 3.7 ± 0.48 | 5.4 ± 0.96 | 5.2 ± 0.78 m |
| Te                     | 3               | 3.8 ± 1.03 | 4.8 ± 1.13 | 5.3 ± 1.25 | 5.3 ± 1.06 m |
|                        | 6               | 3.8 ± 0.91 | 5.1 ± 0.73 | 5.5 ± 0.85 | 5.3 ± 1.06 m |
|                        | 9               | 4.3 ± 0.94 | 5.1 ± 0.56 | 5.6 ± 0.84 | 5.4 ± 0.96 m |
|                        | 12              | 4.0 ± 1.05 | 5.5 ± 0.70 | 5.6 ± 0.84 | 5.7 ± 0.67 m |
|                        | 15              | 4.8 ± 0.91 | 5.6 ± 0.51 | 5.6 ± 0.56 | 5.7 ± 0.48 m |

Means (± SD) values followed by the same letter in the same column are not significantly different 5% Duncan's Multiple Range Test. Ro: fermentation using baker’s yeast; Ta: fermentation using tape’s yeast; Te: fermentation using tempeh’s yeast.

3.3. Chemical analysis of virgin coconut oil
Water content, free fatty acids, and peroxide value are interrelated, where the increase of water content will increase oil-free fatty acids, which easily oxidized than their ester form. Table 3. showed that interaction of type and yeast concentration has a significant effect on VCO water content, which generally the increasing of yeast concentration will increase the VCO water content. This is in accordance with the statements of [15] that the increasing of yeast concentration will increase the VCO water content due to the accumulation of water content of yeast starter and coconut oil in the ingredients. The highest water content of 0.136% was found in baker’s yeast treatment with 15% yeast concentration but was not significantly different compared to water content in tape (Ta) and tempeh (Te) yeast treatment. Water content is an important parameter used to determine the quality of the VCO produced while at the same time affecting the shelf life. The higher water content of VCO causes the oxidation process to produce rancidity [16].

Table 2. The organoleptic test result of virgin coconut oil (VCO) for 24 hours fermentation
Different from water content, the yeast concentration has no significant effect on free fatty acid of VCO, but from Table 3 it is known that generally higher yeast concentration produces a higher free fatty acid. The increase of VCO free fatty acid content is an indicator of oil damage. [17] stated that one of the factors that causing oil and fat damage is the enzymatic process that occurs mainly due to the activity of water, enzymes, and microorganisms. Higher yeast concentrations increase the population of microorganisms in the material but not significant in increasing the free fatty acid of VCO. This is due to the breakdown of glycerides by microorganisms to produce free fatty acids that require specific environmental conditions such as the availability of light, high temperatures, and oxygen (O₂).

The degree of damage to oil can be determined based on the peroxide number. The higher the peroxide value then, the higher the oil damage. Table 3 showed that the interaction of type and yeast concentration does not significantly affect the peroxide value. Referred to the water content, free fatty acids, and its peroxide value concluded that coconut oil produced at the interaction of type and yeast concentration entirely passes the national standard of SNI 7381: 2008 as pure coconut oil but have not passed the international standard of [18] because the water content in all coconut oil exceeds the maximum allowed. The maximum limit of water content permitted by APCC international standards is 0.1%, while the water content for all coconut oil produced is 0.11 - 0.13%.

### Table 3. Virgin coconut oil (VCO) quality test results of water content, free fatty acid (FFA), and peroxide value of 24 hours fermentation method

| Fermentation Treatment | Concentration (%) | Water Content (%) | FFA (%) | Peroxide Value (meq/kg) |
|------------------------|-------------------|-------------------|---------|------------------------|
| Ro                     | 3                 | 0.113 ± 0.0115<sup>a</sup> | 0.157 ± 0.0058<sup>b</sup> | 1.3296 ± 0.0069<sup>a</sup> |
|                        | 6                 | 0.130 ± 0.0066<sup>cde</sup> | 0.163 ± 0.0058<sup>b</sup> | 1.3183 ± 0.0079<sup>a</sup> |
|                        | 9                 | 0.133 ± 0.0058<sup>cde</sup> | 0.167 ± 0.0058<sup>a</sup> | 1.3092 ± 0.0148<sup>a</sup> |
|                        | 12                | 0.133 ± 0.0058<sup>cde</sup> | 0.163 ± 0.0058<sup>a</sup> | 1.3188 ± 0.0078<sup>a</sup> |
|                        | 15                | 0.136 ± 0.0058<sup>c</sup> | 0.170 ± 0.0006<sup>a</sup> | 1.3213 ± 0.0076<sup>a</sup> |
| Ta                     | 3                 | 0.113 ± 0.0058<sup>a</sup> | 0.160 ± 0.0006<sup>a</sup> | 1.3167 ± 0.0072<sup>a</sup> |
|                        | 6                 | 0.116 ± 0.0058<sup>b</sup> | 0.163 ± 0.0058<sup>a</sup> | 1.3183 ± 0.0059<sup>a</sup> |
|                        | 9                 | 0.113 ± 0.0058<sup>b</sup> | 0.163 ± 0.0058<sup>a</sup> | 1.3254 ± 0.0007<sup>a</sup> |
|                        | 12                | 0.113 ± 0.0058<sup>b</sup> | 0.170 ± 0.0006<sup>a</sup> | 1.3296 ± 0.0069<sup>a</sup> |
|                        | 15                | 0.130 ± 0.0066<sup>cde</sup> | 0.170 ± 0.0006<sup>a</sup> | 1.3338 ± 0.0076<sup>a</sup> |
| Te                     | 3                 | 0.120 ± 0.0066<sup>ab</sup> | 0.160 ± 0.0006<sup>a</sup> | 1.3208 ± 0.0072<sup>a</sup> |
|                        | 6                 | 0.123 ± 0.0058<sup>bcd</sup> | 0.163 ± 0.0058<sup>a</sup> | 1.3133 ± 0.0007<sup>a</sup> |
|                        | 9                 | 0.113 ± 0.0058<sup>b</sup> | 0.167 ± 0.0058<sup>a</sup> | 1.3183 ± 0.0063<sup>a</sup> |
|                        | 12                | 0.123 ± 0.0058<sup>bcd</sup> | 0.163 ± 0.0058<sup>a</sup> | 1.3188 ± 0.0063<sup>a</sup> |
|                        | 15                | 0.136 ± 0.0058<sup>cde</sup> | 0.163 ± 0.0058<sup>a</sup> | 1.3213 ± 0.0065<sup>a</sup> |

SNI 7381:2008

|        | Max | FFA (%) | Peroxide Value (meq/kg) |
|--------|-----|---------|-------------------------|
| Ro     | 0.20 | 2.0     |
| Ta     | 0.20 | 2.0     |
| Te     | 0.20 | 2.0     |

APCC:2009

|        | Max | FFA (%) | Peroxide Value (meq/kg) |
|--------|-----|---------|-------------------------|
| Ro     | 0.10 | 2.0     |
| Ta     | 0.20 | 2.0     |
| Te     | 0.20 | 2.0     |

Means (+ SD) values followed by the same letter in the same column are not significantly different 5%

Duncan’s Multiple Range Test. Ro: fermentation using baker’s yeast; Ta: fermentation using tape’s yeast; Te: fermentation using tempeh yeast.

3.4. *Virgin coconut oil fatty acid analysis*

The results of VCO fatty acids composition testing using yeast Ro, Ta, and Te of 15% concentration with 24 hours fermentation showed the produced VCO fatty met the Indonesian Standard National 7381:2008 and the APCC international standard for virgin coconut oil. The quality of VCO can be determined based on the content of its saturated fatty, especially medium-chain fatty acids (MCFA), that related to the function of the MCFA as an energy source and increase body vitality [15]. The fatty acids produced in VCO are medium-chain fatty acids (MCFA) that are easily digested and oxidized by the body, thus preventing accumulation in the body [11]. If accumulated, the medium-chain fatty acid (MCFA) composition of VCO fermented using baker’s, tape, and tempeh yeast respectively are 59.13,
60.64, and 60.27%. These results indicate that VCO produced using tape yeast (Ta) fermentation has better quality based on the content of its fatty acids.

**Table 4.** Virgin coconut oil (VCO) quality test results of free acids composition

| No | Fatty Acid Types | SNI 7381:2008 | APCC:2009 | Composition (%) |
|----|------------------|---------------|-----------|-----------------|
|    |                  | %             | Ro        | Ta              | Te              |
| 1  | Caproic acid (C6:0) | < 0.7        | 0.10-0.95 | 0.3082          | 0.4652          | 0.3426          |
| 2  | Caprylic acid (C8:0) | 4.6 - 10.0   | 4-10      | 5.3233          | 6.7157          | 5.7812          |
| 3  | Capric acid (C10:0) | 5.0 - 8.0    | 4-8       | 5.1693          | 5.5157          | 5.3805          |
| 4  | Lauric acid (C12:0) | >45.1        | 45-56     | 48.3371         | 47.9487         | 48.7666         |
| 5  | Myristic acid (C14:0) | 16.8 - 21   | 16-21     | 20.4790         | 19.7855         | 20.1455         |
| 6  | Palmitic acid (C16:0) | 7.5 - 10.2  | 7.5-10.2  | 9.7615          | 9.2814          | 9.4286          |
| 7  | Stearic acid (C18:0) | 2.0 - 4.0    | 2-4       | 3.5335          | 3.6561          | 3.3700          |
| 8  | Oleic acid (C18:1) | 5.0 - 10.0   | 4.5-10    | 5.7802          | 5.4128          | 5.5312          |
| 9  | Linoleic acid (C18:2) | 1.0 - 2.5   | 0.7-2.5   | 1.1791          | 1.0949          | 1.1317          |
| 10 | Aracisic acid (C20:0) | -            | -         | 0.0916          | 0.0891          | 0.0870          |
| 11 | Eicosenoic acid (C20:1) | -           | -         | 0.0372          | 0.0349          | 0.0352          |

4. Conclusion

Virgin coconut oil (VCO) using a baker's yeast starter with a concentration of 15% and 24-hours fermentation are the best treatment which produces the highest yield of VCO (13.60%) and is preferred by panellists because it has distinctive taste and aroma of coconut, clear colour and clean appearance. Virgin coconut oil (VCO) that was produced at all types and yeast concentration interaction met the SNI 7381:2008 but failed to meet the APCC international standard related to water content value that exceeds the maximum limit. Free acids composition of virgin coconut oil (VCO) fermented using all types of yeast met the national and international standards, which are SNI 7381:2008 and APCC:2009. Highest lauric acid produces by tempah yeast, while the highest medium fatty acid that is good for health was produced by tape yeast with a composition of 60.64%.

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