

# Extraction of Coconut Oil (*Cocos nucifera* L.) through Fermentation System

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## ABSTRACT

Coconut oil (*Cocos nucifera* L.) has a unique role in the diet as an important physiologically functional food. The health and nutritional benefits that can be derived from consuming coconut oil have been recognized in many parts of the world for centuries. There are few techniques for coconut oil extraction, such as physical, chemical, and fermentation or enzymatic processes using microbial inoculum as enzymatic starter. Starter with different concentration (1.0; 2.5; 5.0; and 10%) of microbial strains were added into coconut cream and allowed to be fermented for over night. The extracted oil was analyzed for further experiment, especially on its antibacterial activity. The maximum yield of 27.2% was achieved by adding 5.0% starter. Water content, acid value, FFA, and peroxide value of the fermented coconut oil were 0.3%, 0.45%, 0.22% and 2.54% respectively. A gas chromatogram showed that this fermented oil contained high lauric acid (46.82%), and 6.01% caprylic, 7.5% capric, 17.02% miristic, 7.21% palmitic, 3.11% palmitoleic, 5.41% stearic, and 1.3% linoleic acid, respectively. An inhibitory effect of such kind coconut oil which contains potential fatty acid against bacterial growth was further examined. It was found that this edible oil exhibited antibacterial activity to inhibit the growth of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescense*, *Bacillus cereus* and *Salmonella*; however it showed slightly inhibitory effect when it was exposed to *Bacillus cereus* and *Escherichia coli*.

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**Key words:** coconut oil, inoculum, fermentation, lauric acid, antibacterial.

## INTRODUCTION

A novel method on improving quality of coconut oil (*Cocos nucifera* L.) to produce best quality of coconut oil for industrial application is increased. One of them is a method of extraction through fermentation or enzymatic system (Rosenthal et al. 1996). Virgin coconut oil (VCO) is made from fresh coconuts, not copra. Since high temperatures and chemicals solvent are not used, the oil retains its naturally occurring phyto-chemicals which produce a distinctive coconut taste and smell. The oil is pure white when the oil is solidified, or crystal clear like water when liquefied. The oil contained high lauric acid (C-12, c.a. 50%) as saturated fatty acid and has known well as medium chain fatty acid (MCFAs). MCFAs are burned up immediately after consumption and therefore the body uses it immediately to make energy rather than store it as body fat (Enig, 1996; Kabara, 1984).

Studies have revealed that populations who traditionally consume large quantities of coconut as a

part of their diet have a low incidence of health problems associated with blood clotting, including heart disease and stroke (Prior et al., 1981). Coconut oil is very stable and does not need to be refrigerated since it contains a saturated fatty acid, because all the carbon-atom linkages are filled or saturated with hydrogen. This means that they do not normally go rancid, even when they heated, degraded, irradiated, oxygenated for cooking or other purposes (Issacs, 1986; Rindengan and Novarianto, 2004).

The VCO processed by fermentation or enzymatic system has more beneficial and safety effect rather than traditional methods from copra, since they often infected by insects or aflatoxin producing molds that caused potential toxicity problem during manufacturing. Traditional coconut oils are considered to be low quality products which indicated by high moisture and free fatty acid content. It was therefore easily to rancid and turned to brown and exhibited relatively short life-time by sensory test (Soeka et al., 2008).

Extraction process of coconut oil through fermentation or enzymatic system involved microbial starter inoculums or enzymatic starter that play a role on breaking of coconut milk emulsion, while through traditional processes the oil extraction were carried out physically by using of heating or mechanical

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expelling (Ketaren, 1986). Activities of enzymes were affected by substrate and enzyme concentration, pH, temperature, and incubation time (Pelczar and Chan, 1986). Microbial starter was utilized as their shown on proteolytic, amylolytic and lipolytic capacities. These kinds of enzymes are required to hydrolyze protein, carbohydrate and lipid components contained in the coconut kernel. Basically, the purpose of the fermentation or enzymatic processes is to make the coconut emulsion into unstable condition and therefore easily to separate into oil phase on upper layer and carbohydrate, protein and water phase on below layer (Soeka et al., 2008; Rahayu et al., 2008).

The purpose of research was to develop the extraction method of coconut oil to produce high quality virgin coconut oil (VCO) and expectedly useful for improving technology on extraction of VCO naturally.

## MATERIALS AND METHODS

### Microbial strains

The strains used in this experiment were *Lactobacillus bulgaricus*, *Saccharomyces cerevisiae*, *Candida rugosa*, *Aspergillus oryzae*, *Salmonella*, *Pseudomonas fluorescens*, *Escherichia coli*, and *Bacillus subtilis*, soy-sauce starter (*Aspergillus oryzae*), bake yeast (*Saccharomyces sp*), tempeh starter (*Rhizopus oligosporus*), and beverage yeast (*Candida utilis*) obtained from the collection of Microbiology Division of Research Center for Biology, Indonesian Institute of Science (LIPI) Cibinong-Bogor.

### Chemical reagents

Bacto-peptone, yeast extract, agar, *potatoes dextrose agar* (PDA),  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , soluble starch were purchased from Sigma and Merck.

### Steps of experiment

The experiment was carried out on five steps, i.e.: (i) strains selection, (ii) starter production, (iii) coconut oil extraction, (iv) qualitative and quantitative analysis of oil product, (v) assay on antibacterial activity of coconut oil.

### Coconut oil preparation

The coconut type used for making virgin coconut oil was according to method of Rindengan and Novariant (2004). The coconut cultivars used in this experiment were kelapa dalam, genjah salak, and genjah kuning. Matured coconut was grated and the grated coconut was then mixed with hot tap water (1:1, w/v). After squeezing and filtering, coconut milk was pooled into a clean jar and stayed for 1h. After separating into two layers those were cream on upper part which riched in oil content while skim layer riched in protein on below part was drained off, and the remained cream one was then fermented overnight to prepare virgin coconut oil.

### Agar media preparation

Potatoes dextrose agar (PDA) media and nutrient agar (NA) media containing 0.75 g yeast extract, 1.25 g peptone, 5g agar and 10g potato or malt extracts, respectively, were prepared according to Cappuccino and Sherman (1983). These ingredients were dissolved into 250 mL distilled water, and then melted using microwave for 3 min to accelerate their solubility. The melted media were poured into tubes and autoclaved for 15 min at 121°C and cooled down onto elevate rack to prepare slant culture media.

### Microbial screening

To prepare enzymatic starter for extracting virgin coconut oil, both of yeast and mold strains were inoculated onto PDA and bacterial strain onto NA and then incubated for 3 days at room temperature. Stock cultures were transferred into liquid media containing coconut water, coconut skim, pineapple or malt extract, urea and molasses.

### Assay for enzymatic activities

Selected media for assaying proteolytic and amylolytic activities was referred to a method of Sulistyono et al. (1999). One ose-needle of stock culture of bacterial strain was inoculated into nutrient broth (NB) and incubated for 24h at 37°C. One mL of culture was added into 9.0 mL of NB media and incubated for 24h at 37°C. One mL of respective stock cultures were inoculated into 9.0 mL NB, and incubated at 37°C for 2 days. Proteolytic activity was measured semi quantitatively on the media containing 1%  $\text{KH}_2\text{PO}_4$ , 2%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1% yeast extract, 5% agar and 2% skim milk. Qualitatively analysis was done based on activity tested on agar media grown with 3 days-old microbial strains. The proteolytic activity was indicated by present of clear zone surrounded colonies of strains. Amylolytic activity was measured as by measuring proteolytic activity when 1% soluble starch was applied to the media rather than 2% skim milk. Observation was carried out on present of clear zone after employing iodine reagent (Mesteky et al., 2004).

### Enzymatic starter preparation

The media for production of starter containing coconut water, coconut skim, pineapple or malt extract, urea and molasses in 500mL Erlenmeyer flask was sterilized using autoclave for 15 min at 121°C. After cooling down to a room temperature, the media were inoculated by *Lactobacillus bulgaricus*, *Aspergillus oryzae*, *Candida rugosa* and *Saccharomyces cerevisiae*, respectively. The respective starters those were incubated with different cultures were then employed to the coconut cream and incubated at 40°C for overnight. The oil was obtained through this process were then measured and analyzed. Influence of strains growth toward incubation temperature at 25, 30, 35, 40 and 45°C and pH of media at 3, 4, 5, and 6 during incubation

on the shaker for 5 days and employed concentration of starter at 1%, 3%, and 5% toward the yield of obtaining coconut oil were studied to determine capacity of respective microbial strains those were suitable for preparing an effective starter in producing high yield of VCO (Sulistyo et al., 1999).

The strain that exhibited high yield on oil production was selected and examined further in comparison to the capacities of commercial starter products on extracting coconut oil through fermentation system, such as soy-sauce starter mold (*A. oryzae*), bake yeast (*Saccharomyces* sp.), tempeh starter (*R. oligosporus*), and alcoholic beverage yeast (*C. utilis*). The obtaining oils derived from different coconut type were then filtered through activated charcoal and analyzed using gas chromatography (GC).

#### Yield measurement

Yield of obtaining coconut oil was determined by using the method of gravimetric (v/v) as follows:

$$\text{Yield} = \frac{\text{Volume of obtaining oil (mL)} \times 100\%}{\text{Volume of coconut cream (mL)}}$$

#### Fatty acid analysis

Sample of VCO was analyzed according to the method of Rietschel et al. (1972). Approximately 20-30 mg of sample was placed into a tube with cap and added with 1.0 mL 0.5 N NaOH in methanol and hydrolyzed for 20 min. After addition with 2.0 mL of 16% BF<sub>3</sub> in methanol and 2.0 mL of saturated NaCl to remove emulsion, the reaction mixture was then extracted with hexane. The hexane layer was then transferred into a flask containing anhydrate 0.1g Na<sub>2</sub>SO<sub>4</sub> as moisture absorbent. The prepared sample was injected onto GC using internal standard of fatty acids, under GC condition at 190-200°C, flow rate 1.0 cm/s, fused silica capillary column (3 m length), flame ionization detector and volume of sample injection was 4.0 µL.

#### Proximate analysis

According to the method of Suminar et al. (2001), the proximate analysis for determining acid value, free fatty acid, moisture and peroxide content of VCO was carried out by preparing 2.5 g of sample onto erlenmeyer flask. A titration reagent of 25 mL alcohol-benzene (1:1, v/v) was pre-heated on a water bath at 70°C for 10 min after addition with 3 drops of phenolphthalein as indicator and the mixture was titrated with 0.01 N NaOH up to the solution just turned to slight red. The solution was mixed with sample and heated for 5 min and titrated again with 0.01 N NaOH at least for 10 min.

$$\text{Acid value} = \frac{A \times N \times 40}{\text{Sample weight (g)}}$$

$$\text{FFA (\%)} = \frac{A \times N \times M \times 100\%}{\text{Sample weight (mg)}}$$

A = Quantity of NaOH  
N = Normality of NaOH  
M = MW of lauric acid

#### Moisture content

Moisture content was determined by weighing 10g of sample and placed onto a petri dish that had already determined for its blank weight. The sample was heated at 105°C for 2h in an oven and cooled it down in a desiccator for approximately 15 min and weighed again.

$$\text{Moisture content} = \frac{A - B}{A} \times 100\%$$

#### Assay on antibacterial activity

Antibacterial activity was assayed by preparing nutrient broth (NB) in some reaction tubes. The media containing 0.3 g yeast extract and 0.5 g peptone in distilled water was sterilized by autoclaving at 121°C for 15 min. The NB media was incubated on the shaker after inoculating with 10se of tested bacteria for 2 days. One mL of pre-incubated media which containing the tested bacteria was then diluted into the tubes containing 9.0 mL of sterilized distilled water and more over diluted gradient up to obtaining dilution at 10<sup>-3</sup>. Finally, 0.1 mL of the 10<sup>-3</sup> diluting sample was transferred onto the petri dish containing NA media. To determine the activity of VCO against bacterial growth, a smeared paper dish with VCO was placed onto the media, and the activity of antibacterial was assayed by observing the present of clear zone surrounding the colony that had grown by tested strains after 2 days incubation (Carson and Riley, 1995).

## RESULTS AND DISCUSSION

#### Amylolytic and proteolytic activity

To determine the capacity of some microbial strains in producing enzymatic starter that was suitable for extraction of VCO, we had employed four selected microbial strains were *L. bulgaricus*, *S. cerevisiae*, *C. rugosa* and *A. oryzae*. The enzymatic activities of these strains were investigated according to the method of gel diffusion on the media containing starch for amylolytic activity or skim milk for proteolytic activity as mentioned in the Methods and Materials. The strain of *L. bulgaricus* showed the highest activity for amylolytic and proteolytic enzymes as indicated by formation of colony surrounding clear zones. Diameter of clear zone (± 2.0 cm) was undoubtedly illustrated that the strain of *L. bulgaricus* capable to produce amylase and protease those were available important to digest protein and carbohydrate which contained in coconut cream as its substrate. The strain *L. bulgaricus* was furthermore selected to be employed as potential starter for extracting VCO, while the other strains, *S. cerevisiae*, *C. rugosa* and *A. oryzae*, respectively. *A. oryzae* had not been

employed as starter since their proteolytic and amylolytic activities given are lower than *L. bulgaricus* (Table 1).

**Table 1.** Proteolytic and amylolytic activities of the selected strains.

| Activity/Strain | Diameter of clear zone (cm) |      |     |     |
|-----------------|-----------------------------|------|-----|-----|
|                 | LB                          | K-1A | SC  | CR  |
| Proteolytic     | 2.0                         | 1.6  | 1.3 | 1.6 |
| Amylolytic      | 2.1                         | 1.4  | 1.4 | 1.5 |

Note: LB: *Lactobacillus bulgaricus*; K-1A: *Aspergillus oryzae*; SC: *Saccharomyces cerevisiae*; CR: *Candida rugosa*.

#### Fermentation system

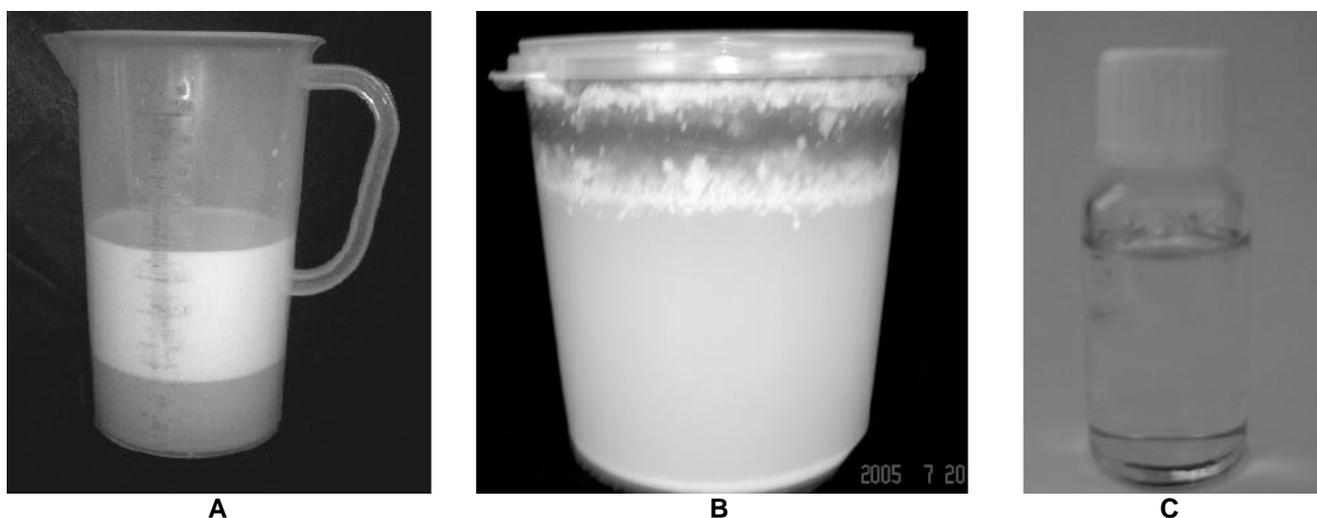
The fermentation of coconut cream occurred when the enzymatic starter had been employed for processing. Crude coconut oil was formed due to a phenomenon of protein digestion that plays a role to stabilize emulsion of the coconut cream into a soluble material. The enzymatic starter with high capacity of amylolytic and proteolytic could hydrolyze carbohydrate and protein which contained in the coconut the cream as its substrate into soluble sugar and amino acid and peptide (Soeka et al., 2008). The extraction process of coconut oil via fermentation or enzymatic system involved microbial cell and enzymes those could solve the emulsion; however, their activities were influenced by some conditions of substrate, enzyme, pH, temperature, and incubation period (Pelczar and Chan 1986).

Preliminary step on extraction process of VCO was initiated after separating the coconut cream which higher in lipid content from coconut skims which higher in carbohydrate and protein content as shown on Figure 1.A. After addition with starter followed by overnight fermentation of the coconut cream at room temperature, the starter containing enzymes were stimulated to digest starch and ferment it into alcohol and organic acids that

coagulate protein in consequence of phases formation of oil on upper part, protein in the middle and water layer on lower part (Rindengan and Novarianto, 2004). Due to a lower molecular weight, the oil part formed through the process could be directly separated from protein and water part by draining off both of them through a valve (Figure 1.B). To reduce interference of water content or insoluble materials into the oil part, a further process of obtaining oil by refining through filter paper or vacuum filter and rinse with hot water following by vacuum evaporation was required to avoid chemically processing to achieve the virgin state of oil as shown on Figure 1.C.

Fermented coconut oil has been known well as virgin coconut oil (VCO) since high temperatures, chemicals or other physical treatment are not used in its processing. As it had been naturally and traditionally processed through enzymatic fermentation, unhydrogenated, undeodorized, and unbleached, the component of fatty acids, especially lauric acid of this coconut oil is not change since it is least vulnerable of all the dietary oils to oxidation and free-radical formation, and it is therefore the safest to use in cooking. It does not become polymerized and form by-products as do other oils when heated to normal cooking temperatures (Kaunitz and Dayrit, 1992; Rindengan and Novarianto, 2004; Sulisty, 2004).

Structurally, coconut oil is very rare amongst all the other dietary lipids. As a different class of saturated fat that behaves very differently in the body from each other, since it is composed almost entirely of medium chain fatty acids (MCFA), a powerful antimicrobial, where mother's milk is very high in them. Coconut oil is composed of an incredible 64% MCFA. The body metabolizes MCFA and absorbed directly from the intestine into the portal vein, and sent straight on to the liver, where they are burned for



**Figure 1.** A. Coconut milk separation, B. Fermentation process, C. Purified coconut oil.

fuel, almost like a carbohydrate. Rather than produce fat, they are used to produce immediate energy. And, the body uses much less energy to digest MCFA. They are easily digested by saliva and stomach enzymes and do not require pancreatic enzymes. This relieves stress on both the pancreas and the digestive system. For this reason, MCFA are *essential* in baby formulas, and are routinely used in hospitals for patients with digestive, metabolic and malabsorption problems. Also, the MCFA in VCO are used to improve insulin secretion and the utilization of glucose, and therefore greatly helps relieve the symptoms and reduce the health risks of diabetes (Enig, 1996).

#### Screening on microbial strains

Influence of employing pH (Figure 2), temperature (Figure 3) and starter concentration (Fig 4) on the yield of extracting coconut oil exhibited that the strain of *L. bulgaricus* could effectively extract the oil higher than the tested microbial strains when the starter was employed to incubate the coconut cream under the fermentation condition at pH 5.0, 45°C and 5% starter concentration.

Figure 2 showed the influence of incubation pH on the yield of extracting oil. It was found that the highest yield of oil (27.0%) could be obtained after incubating the starter at pH 5.0. It is probably that the strain of *L. bulgaricus* which employed as the starter is a facultative aerobic strain that had optimal proteolytic capability at pH 5.0. This pH value is affected on occurring toward substrate binding enzyme since the concentration of  $H^+$  potentially affected a linkage between active site of enzyme and its substrate which led to conform the active site into optimal condition for binding the substrate based on the principal of Lock and Key.

Figure 3 showed the influence of incubation temperature on the yield of extracting oil. It was found that the highest yield of oil (27.2%) could be obtained after incubating the starter at 45°C. It is probably that the strain of *L. bulgaricus* behaves at optimal condition between 40-45°C. The binding of enzyme to its substrate and rising temperature up to a certain degree had increased kinetic energy and promoted movements of reacted molecules. It was therefore increasing bumping occurrence between enzyme and its substrate optimally. The enzyme exhibited its activity at certain optimal condition of temperature, and therefore when the temperature is over than its optimal condition, the enzyme would certainly be denaturated.

Figure 4 showed the influence of starter concentration on the yield of extracting oil. It was found that the highest yield of oil (26.8%) could be obtained after incubating the starter at 5.0% (v/v). At low concentration rate of reaction was too low, however, the rate would be higher as increasing of substrate concentration which catalyzed by the enzyme. Increasing of enzyme is not effective when

the concentration of substrate achieves optimal condition since the enzyme is saturated by the substrate binding enzyme complexes.

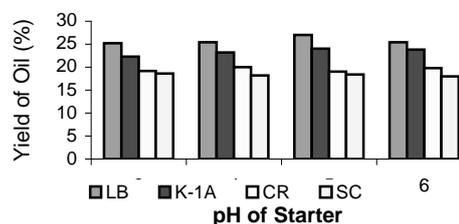


Figure 2. Effect of pH of starter on yield of extracting oil.

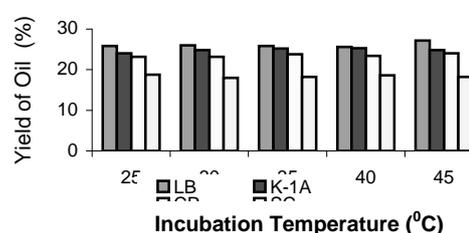


Figure 3. Effect of temperature on yield of extracting oil.

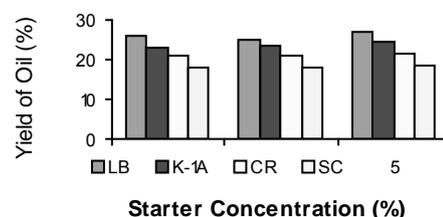


Figure 4. Effect of starter concentration on yield of extracting oil.

Moreover the starter capability of *L. bulgaricus* in extracting of VCO was investigated furthermore in the comparison to other strains contained in soy-sauce starter mold (*A. oryzae*), bake yeast (*Saccharomyces sp*), tempeh starter (*R. oligosporus*), and alcoholic beverage yeast (*C. utilis*). To produce kinds of VCO products, the coconut milk was prepared by different strain of coconut cultivars such as kelapa dalam of Cianjur Regency, genjah salak and genjah kuning of Bogor Regency. The yield of oil derived from each types of coconut were then purified by filtration and absorption using absorbent of activated charcoal as shown on Table 2.

The Table showed that the highest yield of oil was obtained by using coconut cream of kelapa dalam

**Table 2.** Yield (mL) of VCO derived from different type of coconut.

| Coconut Cultivars | VCO starter | Kecap starter | Tempeh starter | Beverage yeast | Bake yeast |
|-------------------|-------------|---------------|----------------|----------------|------------|
| Kelapa dalam      | 25.5        | 28.0          | 21.0           | -              | -          |
| Genjah salak      | 21.5        | 23.0          | 18.0           | -              | -          |
| Genjah kuning     | 15.5        | 17.0          | 15.0           | -              | -          |

Note: (-) none oil formed after processing.

(24.83 mL) while using genjah salak and genjah kuning gave lower yield of oil was 20.83 mL and 15.83 mL, respectively. It was apparent that initial composition of coconut cream significantly influence on final yield of extraction oil. The coconut cream of kelapa dalam exhibited calories (359 cal) and lipid by means of oil content (34.7%) higher than both of the genjah types of coconut cultivar those were 180 cal and 13.0% on the average (Palungkun, 1993). It was found that the oil layer was formed on the coconut cream was incubated by the starter of *L. bulgaricus*, *A. oryzae* of soy-sauce and *R. oligosporus* of tempeh inoculum, while there was none of oil layer found on the coconut cream was incubated by *Saccharomyces* sp. of bake and *C. utilis* of beverage starters. Apparently, the enzyme of these kinds of starters had been inactivated or not appropriated as the starter for enzymatic fermentation process of coconut oil, and resulted in none oil formation since the coconut cream as the substrate was not agreed with the enzymes contained in such starters.

#### Fatty acid analysis of VCO

To determine fatty acid composition of the virgin coconut oil (VCO) obtained by the enzymatic fermentation, sample of the oil was then analyzed by using GC. It was found that the highest yield of lauric acid of oil (42.95%) was obtained by employing the starter of *L. bulgaricus* into coconut cream derived from kelapa dalam strain as shown on Table 3.

**Table 3.** Analysis of lauric acid of extracting VCO.

| Fatty Acid  | Lauric acid concentration (%) |       |       |       |       |
|-------------|-------------------------------|-------|-------|-------|-------|
|             | VCO-LIPI                      | AO-D  | LB-D  | AO-GS | LB-GS |
| Lauric acid | 46.82                         | 41.46 | 42.95 | 40.68 | 35.08 |

Table 3 showed that the VCO obtained by employing the starter of *L. bulgaricus* into coconut cream of kelapa dalam (LB-D) yielded higher lauric acid content (42.95%) rather than by employing the starter of *A. oryzae* with strain of kelapa dalam (AO-D, 41.46%) and the starter *A. oryzae* with the strain of genjah salak (AO-GS, 40.68%) or the starter of *L. bulgaricus* with the strain of genjah salak (LB-GS, 35.08%). It was found somehow; the yield of obtaining oil still lower yet rather than the oil had been obtained by using the starter of VCO-LIPI as the standard of comparison of previous experiment

(Soeka et al., 2008).

#### Quantitative analysis of VCO

To determine the content of FFA, moisture content, peroxide value, the extracting VCO obtained by using of the starter of *L. bulgaricus* was then analyzed and showed as Table 4.

**Table 4.** Analysis of VCO according to SII. 0150-72 for edible oil.

| Component of Analysis | Reference Value of SII | Concentration (%) |
|-----------------------|------------------------|-------------------|
| Moisture content      | Max 0.5 %              | 0.30              |
| Acid value            | Max 0.5 %              | 0.45              |
| Free fatty acid       | Max 2.5 %              | 0.22              |
| Peroxide value        | Max 3.0 %              | 2.54              |

Table 4 showed that characteristic of obtaining VCO for edible oil referred to the moisture content, acid value FFA and peroxide values was agreed with a range of value in accordance to the Standard International of Indonesia (SII) 0150-72 for edible oil. It was suggested that our VCO which was extracted through enzymatic fermentation process by employing the starter of *L. bulgaricus* was appropriated to be consumed as safety and healthy edible oil. The quality of the obtaining oil was corresponded to requirement for quality standard of good edible oil. One of spoiled edible oil indicator is high in acid and peroxide values, since their existences in the product indicate an alteration caused by oxidation on chemical content is being occurred and resulted frequently in a problem of rancid.

#### Assay of antibacterial activity

The antimicrobial properties lauric acid and its derivative monolaurin from coconut oil have shown promise in this study. Lauric acid, which is present in high concentration in coconut oil, forms monolaurin in the animal body and this derivative of lauric acid can inhibit the growth of pathogenic microorganisms (Kabara, 1984). The research focused on *Pseudomonas fluorescense*, *Bacillus subtilis*, *Salmonella* and *Escherichia coli*. To determine potential of lauric acid contained in this extracting oil obtained through enzymatic fermentation, this oil was then studied furthermore against microbial growth. Its antimicrobial activity was observed by the existence of clear zone formed surrounding paper-disc that had pre-submerged into this oil on the media grown with colonies of tested microbial strains. It was found that the clear zone surrounding the paper disc on the media fully grown with strain of *Salmonella*, indicated that this oil had activity against the growth of tested strain as shown on Figure 5. It is now clear and scientifically validated that the inclusion of coconut oil in the diet could and should be utilized for its preventive and healing properties.



**Figure 5.** Assay of antibacterial activity of VCO. (1), VCO-LB; (2), VCO-CR; (3), Copra oil; (4), Palm oil; (5), traditional coconut oil.

### CONCLUSION

The strain of *Lactobacillus bulgaricus* could effectively extract the virgin coconut oil higher than the other tested microbial strains when it was employed into the coconut cream under the enzymatic fermentation condition at pH 5.0, 45°C and 5% starter concentration. The highest yield of oil was obtained by using coconut cream derived from coconut strain of kelapa dalam while using genjah salak and genjah kuning gave only lower yield. It was found that the highest lauric acid (42.95%) was obtained by employing the starter of *L. bulgaricus* into coconut cream of kelapa dalam strain. Characteristic of obtaining VCO as edible oil that had been referred to the moisture content, FFA, acid and peroxide values was in a good agreement in accordance to the Standard International of Indonesia (SII) 0150-72. To determine potential of lauric acid contained in this oil, the study was focused on some microbial strains and It was found that there were clear zone surrounding the *paper disc* after submerging into this oil, onto agar media grown with strain of *Salmonella*, indicating that this oil exhibited activity against the growth of the tested microbial strain.

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