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 fluorescence, Bacillus cereus and Salmonella; however it showed slightly inhibitory effect when it was exposed to Bacillus cereus and Escherichia coli.

CHAPTER ONE

1.0 INTRODUCTION

Coconut oil is an edible oil that has been consumed in tropical countries for thousands of years. As it has a long shelf life and a melting point of 76 °F, it is used in baking industries. A negative campaign against saturated fats in general, and the tropical oils in particular, led to most food manufacturers abandoning coconut oil in recent years in favor of hydrogenated polyunsaturated oils, particularly soy, which contain trans fatty acids. Studies done on populations consuming diets high in coconut oil show no adverse effects on the health of the population.

Coconut oil has >90% saturated fatty acids, hence is less attractive to consumers. Saturated fat is one that has no unsaturation or double bonds and tends to be solid at room temperature. Coconut oil is rich in short and medium chain fatty acids. Shorter chain length allows fatty acids to be metabolized without use of the carnitine transport system.

Various fractions of coconut oil are used as drugs. Butyric acid is used to treat cancer, while lauric acid is effective in treating viral infections. Hence, the literature is reviewed in the context of increase of fat related disorders / diseases through consumption of highly unsaturated oils.

1.2 USES OF COCONUT OIL

There are numerous uses of Coconut Oil. Coconut oil is one of the few herbal internally and externally supplements. It is known of its health benefits which it is used in heart-related problems which includes lowering of triglycerides and cholesterol level. Coconut oil contains Lauric acid which is found in mother’s milk that helps the newborn’s body to fight against various diseases and helps to build their immune system. Once our body digests the Lauric acid, it is transformed into Monolaurin acid which is known for its antiviral, antibacterial, antiprotozoal, antifungal and antimicrobial properties and its action against lipid-coating of viruses. It also contains other important acids such as myristic acid, palmitic acid, caprylic acid, capric acid, stearic acid, linoleic acid, palmitoleic acid and oleic acid.

Coconut oil is also known for its effective use in healing injuries, internally as well as externally. From ancient times, Coconut oil is used as a protective and healing supplement for injuries. Coconut oil can also be used as a moisturizer for all skin types, especially dry skin and aging skin, leaving skin refreshed and looking wide-awake. Moreover, it can be used as hair care product where it provides essential proteins required for nourishing damaged hair.

1.2.1 PRODUCTION METHODS OF COCONUT OIL

One of the most widely used method in treating the oil-in-water emulsion in the production of Coconut oil is the traditional fermentation method. This method is the natural separation of the coconut oil from water using gravity, thus no machine or any other substances are used in the extraction. First, the coconut milk is obtained from the fresh mature coconuts without adding any chemicals. The milk is then fermented in containers for approximately a day. After which, the coconut oil is then separated from the water.

This oil is then carefully filtered to obtain the pure coconut oil and what is called the Virgin Coconut Oil (VCO) (N.A. Nik Norulaini et at., 2009). There are several methods used for the production of coconut oil, such as electrostatic coalescence and centrifugal methods. The existing process production of Virgin Coconut Oil basically is conducted through oil separation from coconut milk (Sukartin and Sitanggang, 2005).

In this research project, the microwave heating and separation technology is used as an alternative method for the production of coconut oil. Electromagnetic radiation in the frequency range 300MHz to 300GHz are known as microwaves, microwave energy is non-iodizing radiations that cause molecular motion by migration of ions and dipole rotations, but does not cause changes in molecular structure and wavelengths ranging from a few centimeters to a few millimeters (Abdurahman et al., 2006). Microwave heating offers a faster processing rate because of its volumetric heating effects. Microwave energy is delivered directly to materials through molecular interaction with the electromagnetic field. In heat transfer, energy is transferred due to its thermal gradients while microwave heating is the transfer of electromagnetic energy to thermal energy and it is more to energy conversion, rather than heat transfer. This difference in the way energy is delivered can results in many potential benefits of using microwaves for the production of virgin coconut oil. The transfer of energy does not rely on diffusion of heat from the surfaces and it is possible to achieve rapid and uniform heating of thick materials (Abdurahman et al., 2006).

1.2.2 SEPARATION PROCESS

In chemistry and chemical engineering, separation process is used to transform a mixture of substances into two or more distinct products. The separated products may differ in chemical properties or physical property, such as size. Almost every element or compound is found naturally in an impure state such as a mixture of two or more substances. Most of the time the need to separate it into its individual components arises. Separation processes can essentially be termed as mass transfer processes. The classification can be based on the means of separation, mechanical or chemical. The choice of separation depends on the pros and cons of each. Usually, mechanical separations are preferred if possible due to its lower cost of operations as compared to chemical separations. Depending on the raw mixture, various processes can be employed to separate the mixtures. Many times two or more of these processes have to be used in combination to obtain the desired separation. In addition to chemical processes, mechanical processes can also be applied where it is possible.

1.3 STATEMENT OF PROBLEM

The tradition method used in the emulsion treatment of the production of Coconut oil is found to be limited and time-consuming. The conventional fermentation method takes longer time to separate the emulsion through the gravity. Gravity is mainly associated with the slow sedimentation process of an immiscible mixture. Sometimes, gravity separation may be too slow because of closeness of the densities of the particles and the fluid, or because of association forces holding the components together (Geankoplis, 2003). Gravity separation takes hours while microwave separation method may be accomplished in minutes or even seconds.

Moreover, the conventional methods for this process may involve the usage of chemicals and high-heating for further refining process, thus causes the loss of natural properties of the coconut oil which further affects the quality of the coconut oil. Furthermore, demand on coconut oil is increasing due to its health benefits. Thus, in my research, combination of microwave and centrifugation method has been discovered as an alternative method to solve these problems which it is believed can helps to save energy and time, also to improve the quality of coconut oil.

1.4 AIMS AND OBJECTIVES OF THE STUDY

Mainly, the objective of this research is focus on the study of the extraction of coconut oil for marketing Distribution potential of combination of microwave and centrifugation separation technology in demulsification (emulsion breaking) of coconut milk emulsion into water and oil. At the same time, comparison between the Combination of microwave and centrifugation separation.

1.5 GLOBAL COCONUT OIL MARKET OVERVIEW

Coconut is a widespread plantation and is grown in more than 80 countries. The world production of coconut has been estimated to be around 55 million tons. in a year. Indonesia and Philippines are the two major coconut producers all across the globe and India captured the third place. The annual coconut production in India is around 2,044 core from 19.8 lakh hectare area. In India, Kerala is the largest producer with the contribution of around 40% of country’s total coconut production. The coconut oil which is extracted from coconut has significant usage in toiletry, food and various industrial sectors. Apart from this, it is also utilized in pharmaceutical industry especially by diabetic patients as it helps in valuable utilization of blood glucose. Besides ordinary coconut oil which is extracted from coconut, there has been an inclination in the demand for virgin coconut oil over the past few years. The virgin coconut oil is generally extracted from fresh coconut milk procured from 12 months of pollination of coconut either by natural or by mechanical techniques. Coconut is rich in fatty acid and includes vitamin, minerals and antioxidants. Coconut oil does not experience chemical processing, or bleaching. It has been notified that the product is gaining traction all over the world as nutraceutical food oil and is getting popular amongst health conscious people.

Coconut oil market is anticipated to expand at a rapid pace over the next few years due to rising public awareness and inclining investment by manufacturers for this product.

1.5.1 MARKET SIZE AND FORECAST

The global coconut oil market captured significant revenue in 2016 and is expected to expand at a CAGR of around 11% over the forecast period. Coconut oil is predicted to experience high demand from consumers with higher health consciousness and as well as aging population. By distribution channel, the online

segment is anticipated to achieve higher market value over the forecast period due to rising internet penetration and availability of large variety of products at a discounted price.

1.6 HEALTH BENEFITS OF COCONUT OIL

According to medical research, coconut oil benefits the body in the following ways:

1. Proven Alzheimer's Disease Natural Treatment

The digestion of MCFAs by the liver creates ketones that are readily accessible by the brain for energy. Ketones supply energy to the brain without the need of insulin to process glucose into energy.

Recent research has shown that the brain actually creates its own insulin to process glucose and power brain cells. As the brain of an **Alzheimer's**patient loses the ability to create its own insulin, the ketones from coconut oil could create an alternate source of energy to help repair brain function.

2. Prevents Heart Disease and High Blood Pressure

Coconut oil is high in natural saturated fats. Saturated fats not only increase the healthy cholesterol (known as **HDL cholesterol**) in your body, but also help convert the LDL "bad" cholesterol into good cholesterols.

By Increasing the HDL in the body, it helps promote heart health and lower the risk of **heart disease**.

Coconut oil also benefits the heart by lowering **high triglycerides**.

3. Treats UTI and Kidney Infection and Protects the Liver

Coconut oil has been known to clear up and heal urinary tract infection (**UTI**) and kidney infections. The MCFAs in the oil work as a natural antibiotic by disrupting the lipid coating on bacteria and killing them. Research also shows that coconut oil directly protects the liver from damage.

Coconut water also helps hydrate and support the healing process. Doctors have even injected coconut water to **clear up kidney stones**. Coconut is a powerful superfood, which is evident given all these tremendous coconut oil benefits.

4. Improves Memory and Brain Function

In a 2004 study published in the *Journal of Neurobiology of Aging*, researchers found that the MCFAs in coconut oil improved the memory problems in their older subjects.

Across all the patients there was a marked improvement in their recall ability after taking this fatty acid. As the MCFAs are absorbed easily in the body and can be accessed in the brain without the use of insulin. Thus, they are able to fuel brain cells more efficiently.

8. Improves Energy and Endurance

Coconut oil is easy to digest and also produces a longer sustained energy and **increases your metabolism**. When taking a quality unrefined coconut oil, you can get the most coconut oil benefits as its MCFAs are sent directly to the liver to be converted into energy.

Today, many triathletes use coconut oil as their source of fuel during training and races for long-distance events. You can make a homemade energy fuel by mixing coconut oil, raw honey and chia seeds together. Simply put together one tablespoon of each and consume 30 minutes prior to exercise.

9. Improves Digestion and Reduces Stomach Ulcers and Ulcerative Colitis

Coconut also improves digestion as it helps the body absorb fat-soluble vitamins, calcium and magnesium.

If coconut oil is taken at the same time as **omega-3 fatty acids**, it can make them **twice as effective**, as they are readily available to be digested and used by the body.

Coconut oil can help improve bacteria and gut health by destroying bad bacteria and candida. Candida imbalance especially can decrease stomach acid, which causes inflammation and poor digestion. All this together means coconut oil benefits digestive health and helps treat or prevent **stomach ulcers** and **ulcerative colitis**.

10. Reduces Symptoms of Gallbladder Disease and Pancreatitis

The MCFAs of coconut oil do not need the pancreatic enzymes to be broken down, so taking coconut oil eases the strain on the pancreas.

Additionally, this superfood is so easy to digest that it has been known to improve the symptoms of **gallbladder** disease as well. Replace other long-chain fats with coconut oil to improve gallbladder and total body health.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Coconut oil contributes about 10% of the total oils and fats in the world market. Several methods are currently practiced in the production of coconut oil either from fresh coconut meat or copra (dried coconut kernel). These technologies include the (i) wet process, (ii) dry process, and (iii) solvent extraction. The recovery of coconut oil by the traditional wet process is usually low, which is about 30% (Thieme et al., 1968). Furthermore, the oil obtained is of poor quality due to the high moisture content (MC), dark color, and short shelf life (Hagenmaier et at., 1973). The process is also energy- and time-consuming.

2.2 WET PROCESS

The wet process is a process where the coconut meat is grinded with water and is then filtered to produce coconut milk or coconut cream (oil-in-water emulsion). This emulsion contains protein and coconut oil which can be separated either through common kitchen utensils or hydraulic presses. Using this process, the coconut oil is extracted from the coconut meat without drying it first. However, coconut oil extraction by these wet process techniques has not been commercially successful.

2.3 DRY PROCESS

The present commercial technique for coconut oil production is through the dry process. Fresh coconut meat is dried first which is then used to press out the oil where the copra is first cleaned, ground, steamed, and pressed through an expeller for coconut oil extraction. This extracted oil is then further purified by neutralization, bleaching, and deodorization to remove free fatty acids, odors, flavors and pigments. Coconut oil has been exclusively marketed using the traditional fermentation method. This method is the natural separation of the coconut oil from water using gravity, no machine or other substances are used in the extraction and therefore it is time-consuming. First, the coconut milk is obtained from the fresh coconuts without adding any chemicals. The milk is then fermented in containers for approximately half a day. After which, the water is separated from the oil where the lighter coconut solids (curds) float to the top while the heavier water sink to the bottom. The coconut oil is in between the curds and the water. This oil is then carefully filtered and separated. The oil is then slightly heated for a short time to drive off excess moisture and produce a more purified product and to extend shelf life. Here, produce the VCO which retains its natural scent and taste of coconuts. This fermentation

method is a traditional method of VCO production that has been used for hundreds of years.

2.4 COCONUT OIL QUALITY

A number of reviews are available on the different types of extraction and processing methods for the preparation of coconut oil, coconut flour, protein and coconut cake and meal. (Cornelius A., 2003) has reviewed the aspects of coconut processing such as growing, harvesting, handling, storage, composition of nut, processing, primary products (copra, ball copra, desiccated coconut, fibre and shells), coconut oil (extraction methods, composition of coconut oil), uses of oil, coconut cake and meal, nutritional aspects, and the coconut industry in the 7 major producing areas (Philippines, Indonesia, India, Sri Lanka, Mexico, West Malaysia, and Papua & New Guinea). (Dendy and Grimwood 2003) have reviewed the various processes for wet treatment which have been proposed for extraction of oil and protein from fresh coconut meat: The Chayen, Robledano, ICAIT, Krauss-Maffei, Roxas and Sugarman processes, integrated processes, methods used by the Texas A&M University and the Tropical Products Institute, London. The problem of obtaining cheap protein-based food products, coconut milk and cream, frozen milk, syrup, etc. is then examined. A few indications are given regarding the nutritive and chemical aspects of coconut protein. Baltasar (19) has reviewed the extraction process of coconut oil by the dry processing technology. Coconut oil extraction employing dry processing technology is discussed under the following headings: preparation of raw material; drying or cooking; feeding the expeller presses; handling and filtering of crude oil; oil cooling system; and extraction by the solvent method. (Loncin, *et al*., 2004) reviewed the utilization of palm oil and coconut oil in the form of interesterified fat. The utilization of transesterified palm oil with 25% coconut oil for production of cooking fat of uniform consistency little affected by temperature. (solid fat index at 10, 20 and 30 degree C being 45.6, 33.6, 9.2, consistency in 0.1 mm ASTM penetration depth after 9 days' storage at 15, 21 and 25 degree C was 19, 38 and 44). Due to its low oxidation during heating, liquid oil is suitable for table and frying use.

2.5 SOLVENT EXTRACTION

Cancel et al (28) has standardized conditions for coconut oil extraction from coconut milk press-cake. (Gonzalez et al 2009) studied the solvent extraction of residual oil from wet coconut meal using isopropanol. (Bernardini 2000) has described a new single solvent direct extraction process (by CMB, Pomezia) which obviates the need for pressing.

(Aliwalas and Buccat 2013) studied the filtration-extraction of granulated coconut on a bench scale. (Claudio et al 2002) carried out laboratory scale studies on the preparation of a highly nutritious coconut flour from granulated coconut. Preliminary feeding expt. indicate a PER comparable with casein. Prepared foods (cakes, doughnuts, cookies, pastries) with 20-30% wheat flour replaced by coconut flour obtained high taste rating.

2.6 FATTY ACID COMPOSITION

(Banzon and Resurreccion 2003) carried out a study on the fatty acid distribution in coconut oil obtained by four processing methods and secured from four Philippine types of coconuts. There was no observed change in the fatty acid distribution in samples of coconut oil obtained by 4 methods, namely: solvent extraction, fermentation, freezethawing and heating. Neither was there such a change observed in coconut oil samples obtained from 4 types of coconuts.

2.7 EDIBLE APPLICATIONS OF COCONUT OIL

Coconut oil has a high degree of saturation with a high content of saturated fatty acids. Because of high content of saturated fatty acids coconut oil is highly resistant to oxidative rancidity, coconut oil is used as a component of infant milk powders because of its easy digestibility and stable flavor. Coconut oil is extensively used in the food industries as a confectionery fat particularly in

the preparation of ice creams. In imitation chocolates coconut oil is used in place of cocoa butter along with cocoa powder.

2.8 EDIBLE APPLICATIONS OF COCONUT OIL

One of the major non-edible applications of coconut oil is in the soap industries; one important chemical derivative of coconut oil is methyl esters of coconut fatty acids, which are produced by treating coconut oil with methyl alcohol. These methyl esters constitutes an important raw material for the chemical industries as they are more stable and are easier to separate by fractional distillation. Coconut oil has many other industrial uses in the pharmaceuticals, cosmetics, plastics, rubber substitutes, synthetic resins etc. Coconut oil has also been found useful for mixing with diesel. These mixture in the proportion as 30:70 has given excellent road performance of diesel vehicles. Methyl esters of coconut oil fatty acids is also being used as lubricants and biodiesel in aviation industry.

2.9 PHYSICO-CHEMICAL CHARACTERISTICS OF COCONUT OIL

2.9.1 SOLUBILITY

Coconut oil is insoluble in water. At temperature above its melting point it is completely miscible with Table 2. Codex standards for coconut oil (5) Characteristic Coconut oil Colour – Platinum cobalt scale (max) 50 Relative density at 40°C/20° C 0.908-0.921 Refractive Index at 40°C 1.448-1.450 Moisture & other volatiles at 105°C 0.1 Free fatty acids, calculated as lauric acid % by mass (max) 0.3 Peroxide value (millequivalents of active oxygen per kg) Not more than 15 Iodine value 6.3-10.6 Sap. value 248-265 Unsaponifiables, % by mass, max g/kg ≤ 15 Reichert value 6 – 8.5 Polenske value 13 –18 REVIEW 18 l July 2010 most of the non-hydroxylic solvents such as light petroleum, benzene, carbon tetrachloride etc. In alcohol, coconut oil is more soluble than most common fats and oils.

2.9.2 CHEMICAL COMPOSITION OF COCONUT OIL

Coconut oil contains a high proportion of glycerides of lower chain fatty acids (Tables 3, 4 & 5). The oil is highly stable towards atmospheric oxidation. The oil is characterized by a low iodine value, high saponification value, high saturated fatty acids content and is a liquid at room temperatures of 27°C.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Fresh coconut and Commercial coconut oil was purchased from was obtained from Ekeonunwa Market Owerri.

3.2 PROCEDURE

3.2.1 EXTRACTION OF COCONUT CREAM

The extraction of coconut milk was carried out as follows: Grated coconut meat and water at 30°C were mixed in proportions of 1:1, 1:2, and 1:3. The mixture was kneaded manually for 5 min, and the milk was extracted, squeezed, and strained through a layer of cheesecloth. The coconut milk obtained was then left to settle for 2, 4, and 6 h. The samples based on the ratio of 1:1 were used to determine the effect of different settling times (2, 4, and 6 h) and temperatures (30, 50, and 70°C) on the oil extraction yield. Coconut milk was then allowed to settle and separate into two layers: the upper cream emulsion layer, which was thick and dense, and the lower aqueous layer, which contained mainly water and was drained off.

3.2.2 TREATMENT OF COCONUT CREAM BY CHEMICALS AND HEAT

The freshly extracted coconut cream was treated chemically as described by Bhowmik and Marth with some modifications. The coconut cream (200 mL), containing 1 g of 30% hydrogen peroxide (H_2O), was incubated at 40°C for 2 h. Catalase (10.5 mg) was then added to decompose the H_2O. Coconut cream was further incubated at 30°C for 3 h. A preliminary experiment was

carried out to determine the degree of stability of the product by a plate count. As a result of the peroxide/catalase treatment, the bacterial plate count was reduced to 3870 cells/mL coconut cream compared to 4.01 × 108 cells/mL coconut cream in the original sample.

3.3 MICROBIAL STRAINS

The strains used in this experiment were Lactobacillus bulgaricus, Saccharomyces cerevisiae, Candida rugosa, Aspergillus oryzae, Salmonella, Pseudomonas fluorescens, Escherichia coli, and Bacillus substilis, 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.

3.3.1 CHEMICAL REAGENTS

Bacto-peptone, yeast extract, agar, potatoes dextrose agar (PDA), KH2PO4, MgSO4.7H2O, 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.

3.4 EXTRACTION OF COCONUT OIL

3.4.1 COCONUT OIL PREPARATION

The coconut type used for making virgin coconut oil was according to method of Rindengan and Novarianto (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.

3.4.2 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 121ºC and cooled down onto elevate rack to prepare slant culture media.

3.4.3 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.

3.4.4 ASSAY FOR ENZYMATIC ACTIVITIES

Selected media for assaying proteolytic and amylolytic activities was referred to a method of Sulistyo et al. (1999). One ose-needle of stock culture of bacterial strain was inoculated into nutrient broth (NB) and incubated for 24h at 37oC. One mL of culture was added into 9.0 mL of NB media and incubated for 24h at 37oC. One mL of respective stock cultures were inoculated into

9.0 mL NB, and incubated at 37oC for 2 days. Proteolytic activity was measured semi quantitatively on the media containing 1% KH_2PO_4, 2% $MgSO_4.7H_2O$, 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 (Mestecky et al., 2004).

3.4.5 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 121ºC. 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 45oC 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 Coconut Oil (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).

3.4.6 FATTY ACID ANALYSIS

Sample of Coconut oil 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% BF3 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 Na2SO4 as moisture absorbent. The prepared sample was injected onto GC using internal standard of fatty acids, under GC condition at 190-200ºC, 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.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 EFFECT OF COCONUT WATER RATIO ON OIL YIELD

The effect of different amounts of water at 30°C added to coconut meat on extraction yield of oil is shown in Table 1. The results show that the 1:1 ratio gave the highest oil yield, compared to 1:2 and 1:3. As the ratio of water added was increased, the oil content decreased. Higher proportions of water increased the dilution effect and therefore decreased the oil yield in coconut cream from 37.09 to 32.50 and 28.48% in 1:1, 1:2, and 1:3, respectively, after 2 h of settling time. The same trend was found for 4- and 6-h settling times. Banzon et al. (1) found

that the composition of coconut cream is largely based on the amount of water added for the extraction of oil. This result showed that adding less water contributed to a higher proportion of oil and is in agreement with the finding of Banzon and co-workers.

4.2 EFFECT OF WATER TEMPERATURE AND SETTLING TIMES ON OIL YIELD.

Water temperature was increased from 30 to 70°C, there was a significantly increased ($P < 0.05$) oil yield for the 1:1 ratio of coconut meat/water when followed by 2–6 h settling times. The oil recovery from coconut cream with 70°C water was 46.23%, while those of water at 30 and 50°C were 37.09 and 40.83%, respectively. The most effective time of settling the coconut milk was 6 h, which yielded 51.33% oil when the water was at 30°C. The yield was further increased to 83.88% with water at 70°C. Therefore, a long settling time is required at higher temperature to efficiently separate the cream emulsion so that higher oil yield is obtained.

4.3 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 Coconut oil 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 coconut oil 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; Sulistyo, 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 anti- microbial, 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 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).

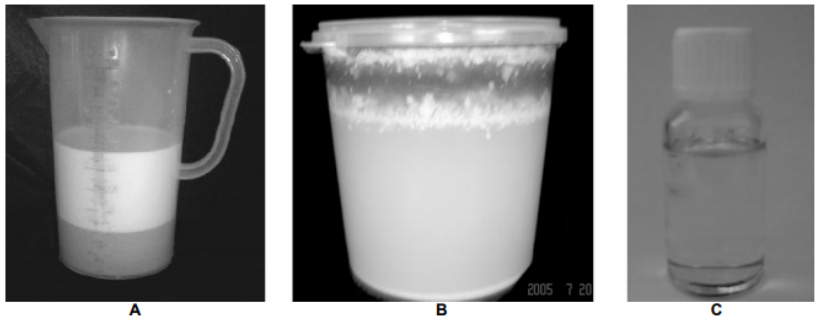

Figure 1. A. Coconut milk separation, B. Fermentation process, C. Purified coconut oil.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This study shows that 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. Extraction of Coconut oil is easily done by fermentation process as discussed in this study. Coconut oil is high in natural saturated fats. Saturated fats not only increase the healthy cholesterol (known as HDL cholesterol) in your body, but also help convert the LDL “bad” cholesterol into good cholesterols. Coconut oil can be extracted and sold in the market due to its health benefits.

RECOMMENDATIONS

The popularity of coconut oil is being seen throughout the natural foods industry, and it’s likely that the consumer demand for this oil isn’t going to drop in the near future. Chances are that if the supply continues to trend downward as it has for the last few years, prices for aren’t likely to drop significantly in the near future. With these, it is recommended that:

- The Ministry of Food and Agriculture to improve upon coconut oil quality and production, in order to meet all standards requirements for local and international markets.
- Institutions such as Environmental Health should enforce good sanitation practises at the processing centres.

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