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Research article

Potential of dragon's blood fruit (*Calamus draco*) as a class I preservative agent and additive ingredient to improve the quality of whole coconut VCO (Virgin coconut oil)

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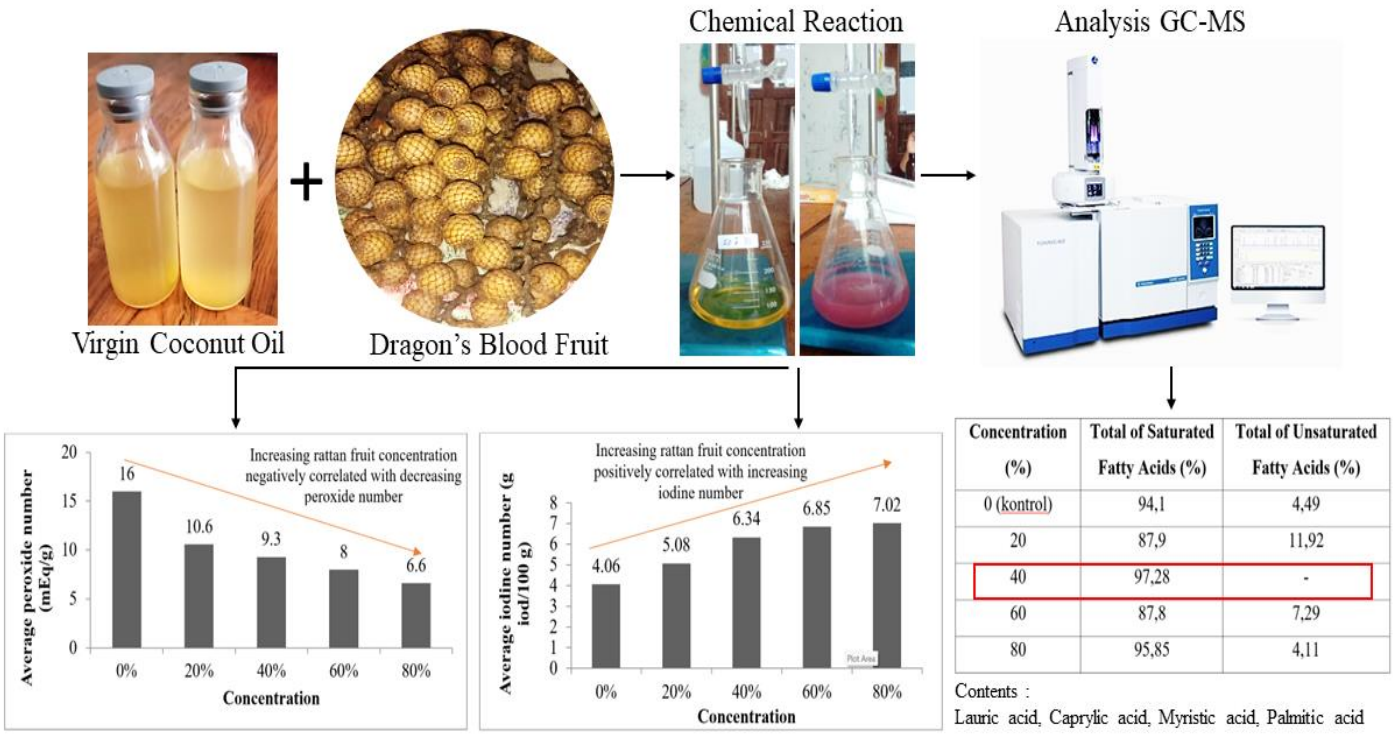
ABSTRACT

The main aim of this research is to develop a method of VCO preservation with dragon's blood fruit addition, which is typical and inexpensive, to obtain good-quality VCO products in terms of fatty acid profile, peroxide, and iodine number.



Concentration (%)	Total of Saturated Fatty Acids (%)	Total of Unsaturated Fatty Acids (%)
0 (kontrol)	94,1	4,49
20	87,9	11,92
40	97,28	-
60	87,8	7,29
80	95,85	4,11

Contents :
Lauric acid, Caprylic acid, Myristic acid, Palmitic acid

The method developed uses whole coconut as the raw material for making VCO through an easy-to-apply fermentation process, adding dragon's blood fruit extract in concentrations of 0%, 20%, 40%, 60%, and 80%. Peroxide and iodine numbers were tested as parameters of VCO quality. The gas chromatography mass spectrometry (GCMS) method was used to analyze the fatty acid profile of VCO after transesterification. The results showed that the highest total saturated fatty acid content of 97.28% was found in the VCO sample with a 40% concentration of dragon's blood fruit, and the unsaturated fatty acid profiles detected in the VCO included palmitoleic acid, methyl linoleic, and methyl oleic. The addition of dragon's blood fruit can also reduce the peroxide number and increase the iodine number in accordance with SNI 7381-2008, which is below the maximum limit of the VCO peroxide number of 2.0 mEq/kg of oil while the iodine number of the VCO is in the range of 4.1–11 g iodine/100g. It can be concluded that a 40% dragon's blood fruit concentration can improve the quality of VCO preservation.

Keywords: Virgin Coconut oil, Calamus draco, Fatty acid profile, Iodine number, Peroxide number.

INTRODUCTION

Virgin coconut oil (VCO) is one of the coconut oil products that is starting to be recognized because it has many health benefits and is useful for raw materials for various industries. For disease treatment, VCO is used to treat HIV-AIDS, cancer, hepatitis, osteoporosis, obesity, skin diseases, and chronic diseases, such as prostate cancer, heart disease, hypertension, and diabetes, as well as various diseases caused by pathogenic microorganisms [1, 2]. Some viral diseases that have been known to be overcome by consuming VCO include avian influenza, HIV/AIDS, hepatitis, and other viral diseases [2]. The existence of these various benefits has caused the demand for VCO to continue to increase both at home and abroad.

VCO is coconut oil made from coconut milk derived from crushed coconut flesh, fermented for several days to form a cream that floats on the top surface. The cream is then heated to extract the oil and isolated. This fermentation process usually involves a succession of microbes and changes in acidity (pH), which will accelerate the oxidation of the oil, causing the oil to become rancid more easily. The high water content in VCO from coconut milk triggers the breaking of the carbon chain into glycerol and fatty acids, which causes the formation of unstable peroxide compounds. These compounds will more easily decompose into volatile compounds with shorter carbon chains such as fatty acids, aldehydes and ketones, causing a rancid odor. Rancidity can reduce the oil's quality and nutritional value, such as the content of vitamins (carotene and tocopherols) and essential fatty acids [3]. Thus, it can be said that the oxidation process can damage its organoleptic and nutritional properties.

To improve the quality and acceptability of VCO, natural ingredients can be added such as dragon's blood fruit, one type of rattan from the largest genus, Calamus. Some types of rattan are usually known for their strength and durability, making them suitable for making various commercial products such as baskets, furniture, sticks, poles, hats, and others [4]. In East Kalimantan, the Dayak people use the genus Calamus, *Calamus manan* Miq or manau rattan in traditional medicine especially to treat mouth ulcers and stomach pain, while *Calamus tenuis* Roxb is used for worms [5, 6]. Other rattan species, such as *Calamus* sp, are known to contain flavonoids and phenolic compounds, with an IC50 value of 6.09 µg/ml, making it a very strong antioxidant [7]. The content of these phytochemical compounds was detected from several parts of the plant, such as pericarp, flesh and seeds. For example, the pericarp and seeds of the rattan species *Calamus manan* Miq, *Calamus caesius* Bl and *Calamus ornatus* Bl contain flavonoids, tannins, and triterpenoids classified as strong antioxidants with IC50 values ranging from 5.42 - 41.17 µg/ml. Meanwhile, the fruit flesh of these species contain tannins and triterpenoids and are categorized as moderate, strong, and active antioxidants, respectively [8]. The antioxidant properties of these various phytochemical compounds are known to counteract and scavenge free radicals, which can not only prevent oxidative stress that induces the emergence of various chronic degenerative diseases but also potentially prevent free radical oxidation in VCO [4, 9-12]. This is due to its ability to donate hydrogen atoms from its hydroxyl group to free radicals so that they are not easily decomposed or oxidized into labile peroxide compounds and will further prevent rancidity[13]. This can then be done as a preservative effort to maintain the quality of VCO oil through the addition of natural ingredients known as class I preservatives. However, the potential of dragon's blood fruit as a class I preservative agent has never been proposed before, so the author considers that there is a need for continuous research efforts to maintain and even improve the quality of VCO through the addition of dragon's blood fruit. Some indicators or test parameters that need to be measured to determine the quality of a VCO product include fatty acid profile, peroxide number and iodine number.

MATERIALS AND METHODS

Materials

The materials used in this study include 22 coconuts were obtained from Peureulak sub-district, East Aceh district, Aceh province, Indonesia; 500 grams of dragon's blood fruit was obtained from Peureulak sub-district, East Aceh District, Aceh Province, Indonesia; n-hexane pro analysis Merck, H_2SO_4 1 M pro analysis Merck, 2 N NaOH pro analysis Merck, methanol pro analysis Merck, distilled water, chloroform pro analysis Merck, glacial acetic acid pro analysis Merck, 0.1 N potassium iodide solution pro analysis Merck, hanus reagent, $Na_2S_20_3$ 0.1 N pro analysis Merck, amylum indicator, and 95% ethanol pro analysis Merck.

VCO Sample Preparation

Virgin Coconut Oil (VCO) samples in this study were extracted from 22 coconuts that have an average weight of 1 kg per fruit so that 200 ml of VCO was obtained. In the process, VCO is made by peeling the coconut and separating the whole coconut from the skin and fiber. Next, the whole coconut is split into two parts to remove the water content, then the two parts of the coconut are put back together by tying it using a rope but leaving a little space, then the coconut is hung in a dark place and not exposed to direct sunlight. Wait for approximately one month for the coconut oil to form. This method is based on traditional methods practiced by traditional healers in the Peureulak region of East Aceh.

Supplementation of Dragon's Blood Fruit (*Calamus draco*)

A total of 500 grams of dragon's blood fruit that has been dried for three days to remove moisture content, coarsely ground and then added to the VCO sample that has been prepared in 5 glass bottles measuring 100 ml (*weight/volume* (w/v)), with various concentrations, namely 0% w/v (100 ml VCO), 20% w/v (20 gr dragon's blood fruit in 80 ml VCO), 40% (40 gr dragon's blood fruit in 60 ml VCO), 60% (60 gr dragon's blood fruit in 40 ml VCO) and 80% (80 gr dragon's blood fruit in 20 ml VCO). Then allowed to stand for four days and filtered using Whatman No.1 filter paper with a pore diameter of 110 mm. VCO samples with dragon's blood fruit combination will then be used for further testing [14].

Determination of Peroxide Numbers

VCO samples as much as 2.5 grams were placed in an erlenmeyer flask with a lid of 250 ml, then added 15 ml of glacial acetic acid:chloroform solution (6:4) and shaken until dissolved. The next step was added 1 ml saturated potassium iodide solution 0.1 N then shaken and allowed to stand for 5 minutes. Then titrated using $Na_2S_2O_3$ 0.1 N until the yellow color in the solution almost disappears (near the end point of titration). Then added 1% amylum indicator as much as 2-3 drops and titrated again with 0.1 N $Na_2S_2O_3$ until the blue color in the solution disappears. The above treatment was done 3 times. The results are expressed in milliequivalents per 1000 grams of oil using the following formula [15].

$$\text{Peroxide Numbers} = \frac{A \times N \times 1000}{G}$$

Description:

A = Amount of $Na_2S_2O_3$ solution (ml)

N = $Na_2S_2O_3$ normality

G = Sample weight (gram)

Determination of Iodine Numbers

Weigh 0.25 grams of VCO sample and put it into an iodine flask. Dissolve the oil with 15 ml of chloroform then add 10 ml of hanus reagent, which has been made before by dissolving 20 ml of iodine-bromide in 1000 ml of glacial acetic acid. Next, the mixed solution was stirred carefully and then kept in a dark place for 30 minutes. Added 10 ml of 0.1 N potassium iodide solution and 50 ml of distilled water that has been boiled before. Titrated iodine solution with $Na_2S_2O_3$ 0.1 N until the color of the solution becomes pale yellow. After that, 2 ml of amylum was added to the solution and continued titration until the blue color disappeared. The above treatment is done 3 times.

The iodine number is the amount (grams) of iodine that can be absorbed by 100 grams of oil. The iod number can express the degree of unsaturation of an oil or fat. The greater the iod number, the higher the degree of unsaturation. Iod number can be calculated through the equation below [16].

$$\text{Iodine Numbers} = \frac{(B-S) \times N \times 12.69}{G}$$

Description:

B = Amount of $Na_2S_2O_3$ 0.1N used in titration blank (ml)

S = Amount of $Na_2S_2O_3$ 0.1N used in sample titration (ml)

N = Normality of $Na_2S_2O_3$ 0.1N after standardization (N)

G = Sample weight (gram)

$$12.69 = \frac{\text{Iodine atomic weight}}{10}$$

Transesterification

A total of 0.5 grams of VCO sample was put into a test tube and added 10 ml of n-hexane then shaken until dissolved. Next, the solution was added 2 ml of 2N NaOH in methanol and vortexed then the solution was heated in a waterbath for 1 minute at 50^0C. The solution was vortexed again and then added 2 ml of 1M H_2SO_4 in methanol. Then the solution is vortexed again and the methyl ester layer is separated. This transesterification process aims to convert triglyceride fatty acids into methyl esters or ethyl esters [17].

Identification of Fatty Acid Using *Gas Chromatography Mass Spectrometry* (GC-MS)

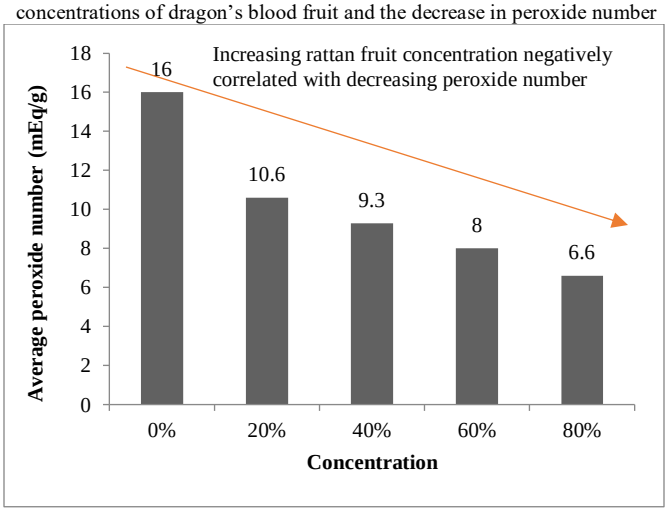
Samples of VCO combined with dragon blood fruit were transesterified and analyzed for fatty acid profile and content using Gas Chromatography Mass Spectrometry (GC-MS) type ISQ 7000 Thermo Scientific with specifications consisting of TG5MS column fused silica capillary column [30 × 0.25 mm ID x 0.25 μm film thickness] at the Pharmacy Laboratory of Syiah Kuala University [17].

RESULTS AND DISCUSSION

Result of Peroxide Number Determination

The peroxide number test is used to determine the degree of rancidity by measuring how many peroxide compounds are formed as a result of fat or oil. From the data of peroxide number measurement, it shows that the addition of dragon's blood fruit can reduce the peroxide number. The relationship between the addition of various concentrations of dragon's blood fruit and the decrease in peroxide number is shown in Figure 1 below.

Figure 1: Graph of the relationship between the addition of various concentrations of dragon's blood fruit and the decrease in peroxide number

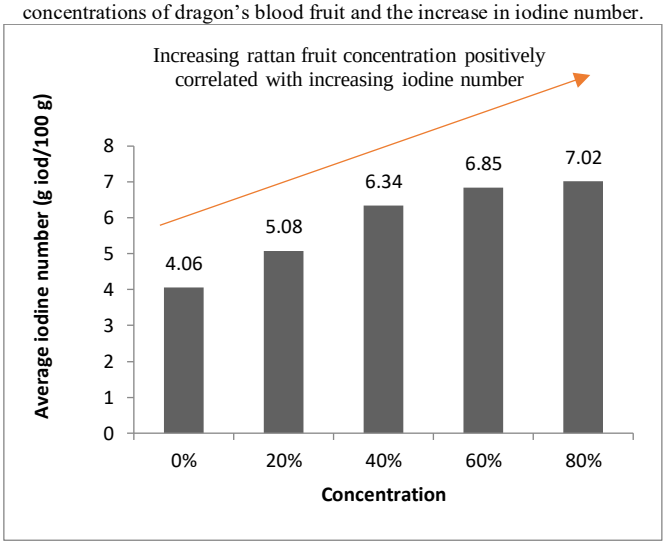


The graph above shows that the higher the concentration of dragon's blood fruit addition to VCO, the lower the peroxide number, which means the better the quality of VCO. In addition, the average peroxide number in VCO samples is in accordance with SNI 7381-2008, which is still below the maximum limit of VCO peroxide number of 2.0 mEq/kg oil. The quality of coconut oil (VCO) is reviewed from the peroxide number because the peroxide number is an important parameter used as a reference in determining the degree of oil damage. Peroxides are formed because unsaturated fatty acids can bind oxygen to their double bonds or what is known as the oxidation process. The oxidation reaction of unsaturated fatty acids can form peroxide compounds. Further degradation of peroxides will form various aldehyde compounds that are volatile and contribute to the formation of rancid odors [18].

Result of Iodine Number Determination

The iodine number is the amount (grams) of iodine that can be absorbed by 100 grams of oil. The iodine number can express the degree of unsaturation of an oil or fat. The greater the iodine number, the higher the degree of unsaturation. The data from the measurement of iodine number shows that the addition of dragon's blood fruit can increase the iodine number, which means the quality of VCO is getting better. The average results of iodine number of VCO samples with the addition of various concentrations of dragon's blood fruit are shown in Figure 2 below.

Figure 2: Graph of the relationship between the addition of various concentrations of dragon's blood fruit and the increase in iodine number.



The graph above shows that the higher the concentration of dragon's blood fruit addition to VCO, the higher the iodine number, which means that the quality of VCO is getting better. In addition, the average iodine number in VCO samples is in accordance with SNI 7381-2008, which is still in the range of 4.1-11 g iodine/100g. Iodine number explains the unsaturation of fatty acids that make up oils and fats. Unsaturated fatty acids are able to bind iodine and form saturated compounds. The amount of iodine bound indicates the number of double bonds contained in the oil [19]. In this case, it can be said that the greater the iodine number, the higher the degree of unsaturation in the VCO sample.

Results from *Gas Chromatography Mass Spectrometry* (GC-MS) instrument analysis

Based on the results of GC-MS analysis of VCO samples added with dragon's blood fruit with concentration variations of 0%, 20%, 40%, 60% and 80%, several types of fatty acids were obtained, including the following Figure 3-7.

Figure 3: Fatty Acid Profiles of VCO + 0% Concentration Dragon's blood fruit

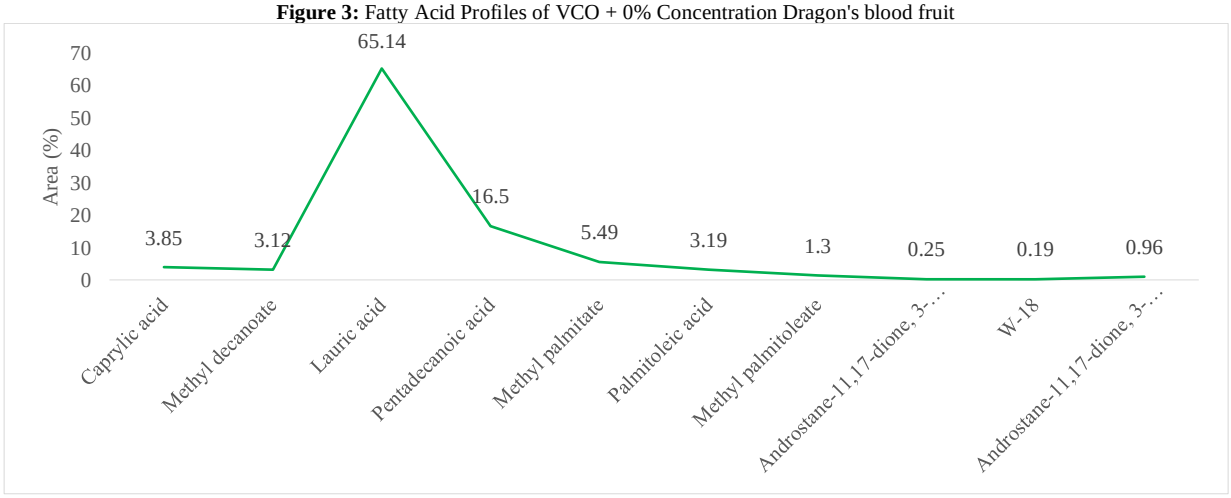


Figure 4: Fatty Acid Profiles of VCO + 20% Concentration Dragon's blood fruit

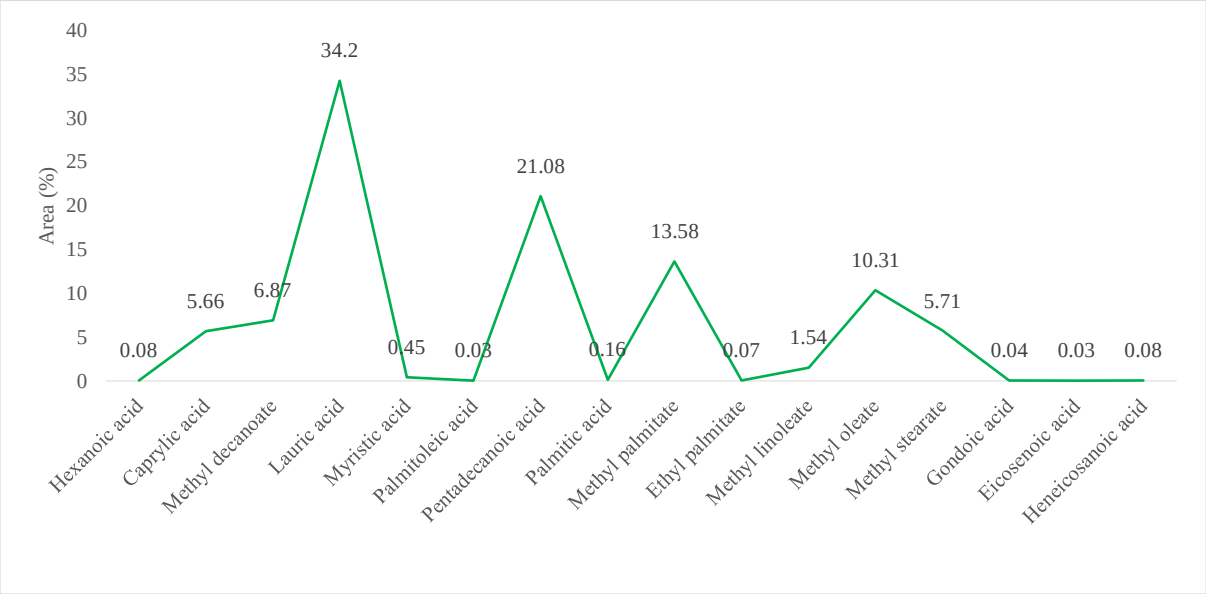


Figure 5: Fatty Acid Profiles of VCO + 40% Concentration Dragon's blood fruit

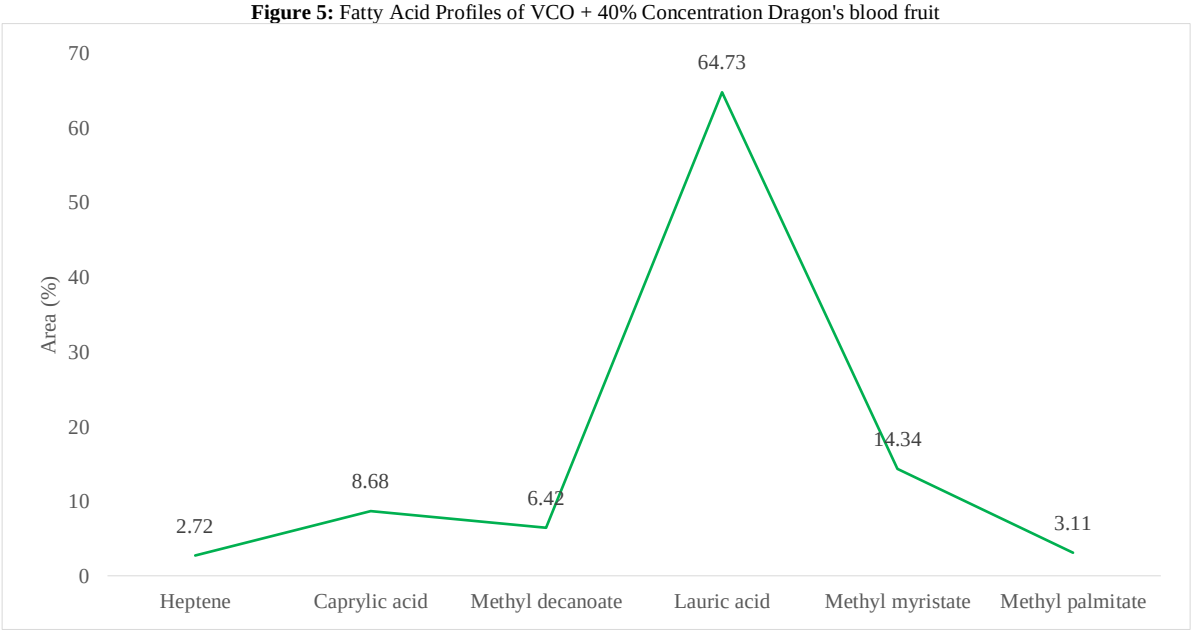


Figure 6: Fatty Acid Profiles of VCO + 60% Concentration Dragon's blood fruit

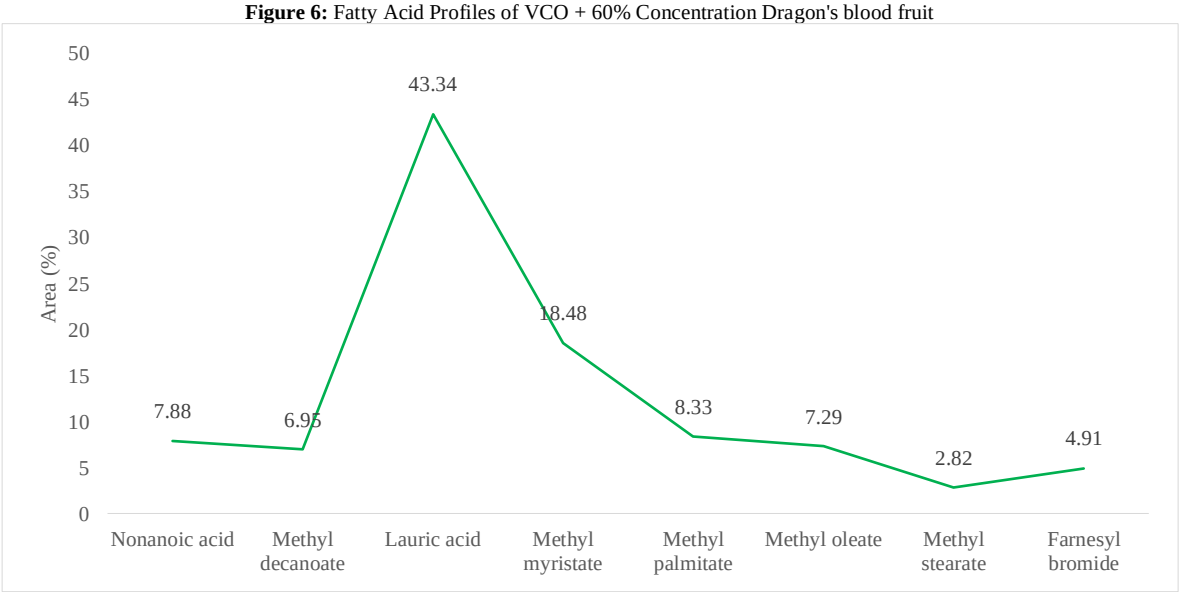


Figure 7: Fatty Acid Profiles of VCO + 80% Concentration Dragon's blood fruit

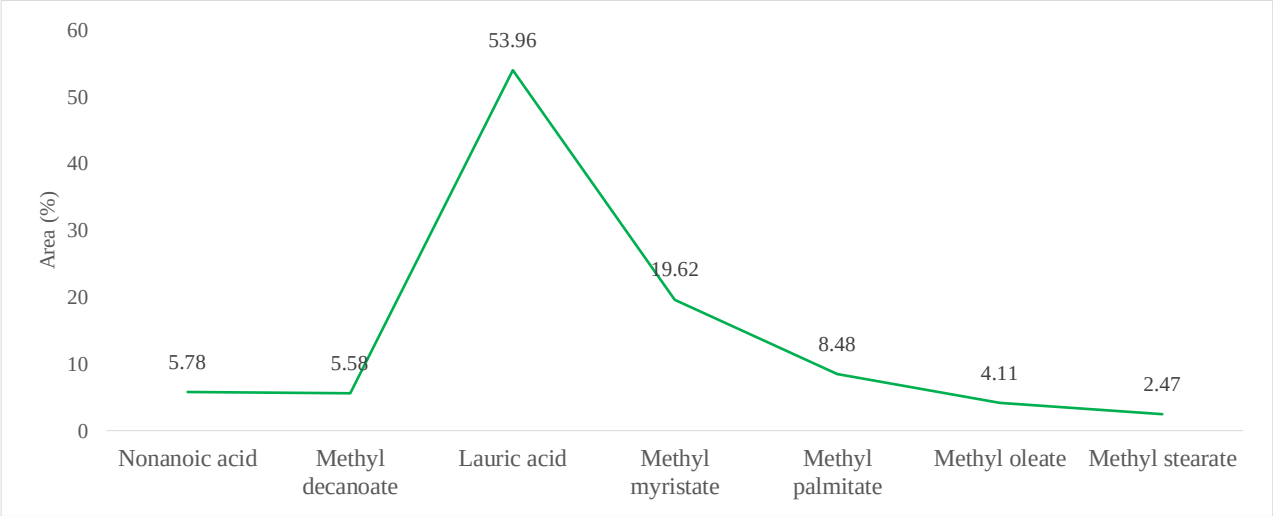


Table 1: Comparison of Dragon's Blood Fruit VCO and Standard VCO in Fatty Acid Profile Test

Test Type	VCO Standard According to SNI 7381-2008	VCO with Dragon's blood fruit Addition				
		0%	20%	40%	60%	80%
Fatty Acid Profile (%)						
Caproic acid	ND-0.7	-	-	-	-	-
Caprylic acid	4.6-10.0	3.85	5.66	8.68	-	-
Capric acid	5.0-8.0	-	-	-	-	-
Lauric acid	45.1-53.2	65.14	34.20	64.73	43.34	53.96
Myristic acid	16.8-21	-	0.45	14.34	18.48	19.62
Palmitic acid	7.5-10.2	5.49	0.16	3.11	8.33	8.48
Stearic acid	2.0-4.0	-	5.71	-	2.82	2.47
Oleic acid	5.0-8.0	-	10.31	-	7.29	4.11
Linoleic acid	1.0-2.5	-	1.54	-	-	-
Linolenic acid	ND-0.2	-	-	-	-	-

Fatty acid profile is a composition of fatty acids that make up a VCO product, consisting of saturated fatty acids (90%) and unsaturated (10%) [20]. The fatty acids contained in coconut oil or VCO are saturated fatty acids which are estimated to be 91% consisting of caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid and unsaturated fatty acids of about 9% consisting of oleic and linoleic. The comparison of VCO with the addition of dragon's blood fruit and SNI 7381-2008 VCO in this fatty acid profile test can be seen in the following Table 1:

Based on the table above, when compared with SNI 7381-2008 VCO quality standards, VCO samples with dragon's blood fruit (*Calamus draco*) supplementation were detected to contain 7 types of fatty acids with different standards consisting of 5 saturated fatty acids including caprylic acid, lauric acid, myristic acid, palmitic acid, and stearic acid and 2 unsaturated fatty acids including oleic acid and linoleic acid. Based on data from fatty acid profile analysis using GC-MS, VCO with 0% concentration contains 5 saturated fatty acids consist of caprylic acid, methyl decanoate, lauric acid, pentadecanoic acid, and palmitic acid, as well as 2 unsaturated fatty acids consist of palmitoleic fatty acid and methyl palmitoleate. The similarity of the types of fatty acids from the SNI VCO quality table with the results of the analysis of 0% concentration VCO is that it contains caprylic acid, lauric acid, and palmitic acid but the intensity is not in accordance with the SNI quality standards due to the content of caprylic acid and palmitic acid detected in the 0% concentration VCO sample which is smaller than the SNI quality of 3.85% and 5.49% respectively. Meanwhile, the lauric acid content in the VCO sample was higher than the SNI quality standard of 65.14%.

Based on the Table 2 above, it is known that the highest composition of VCO is saturated fatty acids with the total content of each sample concentration of the combination of VCO and dragon's blood fruit, namely 94.1%, 87.9%, 97.28%, 87.8% and 95.85%. It can be seen that the most dominant fatty acids in VCO are saturated fatty acids, especially medium chain saturated fatty acids so that whole coconut VCO oil is categorized as medium chain oil (fatty acids consisting of 8 to 12 carbons). Meanwhile, unsaturated fatty acids detected in VCO samples are oleic acid, palmitoleic acid and linoleic acid which have double bonds [17].

Table 2: Composition of VCO

Concentration (%)	Total of Saturated Fatty Acids (%)	Total of Unsaturated Fatty Acids (%)
0 (control)	94,1	4,49
20	87,9	11,92
40	97,28	-
60	87,8	7,29
80	95,85	4,11

Oleic acid and linoleic acid are types of unsaturated fatty acids found in VCO. The unsaturated nature of oil is caused by the presence of double bonds, which in turn will affect the oxidation reaction that causes rancidity [21]. SNI 7381-2008 provides requirements for oleic acid content in VCO samples which is between 5-10% and for linoleic acid which is between 1-2.5%. From the results of identification using GC-MS, VCO samples with the addition of dragon's blood fruit concentrations of 20%, 60%, and 80% contained oleic acid levels of 10.31%, 7.29%, and 4.11%, respectively, while the highest linoleic acid content was found in VCO samples with the addition of dragon's blood fruit concentrations of 20%, which amounted to 1.54%. These results can be said to have met the requirements of SNI 7381-2008. The presence of saturated fatty acids, namely lauric acid, in high quantities was detected in each VCO sample with various dragon's blood fruit concentrations. The highest lauric acid composition was detected in VCO samples without the addition of dragon's blood fruit and VCO samples added with dragon's blood fruit at 40% concentration. However, the fatty acid content in the VCO sample with the addition of dragon's blood fruit at 40% concentration was higher, including caprylic acid, lauric acid, myristic acid and palmitic acid according to SNI standards.

DISCUSSION

The decrease in peroxide number in VCO samples added with dragon's blood fruit (*Calamus draco*) is thought to be caused by the strong antioxidant properties of dragon's blood fruit (*Calamus draco*), which can be attributed to its ability to capture free radicals produced during the propagation stage of the oil by donating hydrogen ions[22]. The ability of antioxidants to donate hydrogen is thought to inhibit the formation of hydrogen peroxide compounds that tend to be unstable and easily decompose into new free radical compounds as a result of the oxidation reaction of unsaturated fatty acids in oil and free radicals [23]. The content of terpenoid compounds contained in dragon's blood fruit is a primary antioxidant that donates hydrogen ions so that it can stabilize free radicals [24]. In other words, the presence of antioxidant compounds in this oil can reduce the speed of the oxidation process that causes rancid odor and taste [25].

In addition, the increase in iodine number in VCO samples added with dragon's blood fruit (*Calamus draco*) is thought to be induced by the activity of antioxidant compounds that can inhibit the bond between hydrogen peroxide compounds formed with double bonds of unsaturated fatty acids through oxidative chain breaking by reacting with free radicals [20]. The high content of antioxidant compounds in dragon's blood fruit is thought to cause the oxidation process to run slowly and inhibit the bond between free radical compounds and double bonds in unsaturated fatty acids so that double bonds in the oil increase and the formation of higher iodine numbers. The higher iodine number contributes to the improvement of oil quality.

Generally, the addition of dragon's blood fruit can be said to have no influence on the percentage of fatty acids in VCO. This is shown by the amount of total saturated fatty acids in each concentration which has increased and decreased so that it cannot be confirmed that the addition of dragon's blood fruit can affect the percentage of fatty acid content. However, it can be observed that the highest composition of saturated fatty acids, lauric acid, was detected in each VCO sample with various dragon's blood fruit concentrations especially in 40% concentration.

Lauric acid is a medium-chain saturated fatty acid with 12 carbon atoms that is found in virgin coconut oil or VCO. Lauric acid has been detected to have antiviral activity that can damage the phospholipid layer of the enveloped viral membrane and further impact the disintegration of the membrane and ultimately trigger the death of the virus. This monolaurin compound is also able to affect the assembly and maturation phases of the viral infection cycle so that the infectious power of this virus is reduced. In addition, lauric acid in VCO also has inhibitory activity against the growth of Escherichia coli bacteria by increasing the diameter of the inhibition zone by 1.7 mm at a concentration of 3.6 mg/ml through enzyme inactivation, protein denaturation, and cell membrane disruption reactions. Meanwhile, in a very small concentration of about 0.062 micromol/ml, lauric acid can inhibit the growth of Pneumococcus bacteria. This compound is also reported to be able to inhibit the growth of *Clostridium difficile* bacteria that cause intestinal dysbiosis, by increasing the formation of free radical compounds of reactive oxygen species that induce damage to the bacterial membrane and ultimately cause the death of the bacterial cells.

It is well known that the presence of some of these microorganisms in foodstuffs, such as VCO, can not only increase the toxic potential of disease but also accelerate the spoilage process and reduce the quality of the material. The antimicrobial properties of lauric acid in VCO can be an important key as a preservative effort in maintaining and maintaining the quality of the oil. This is thought to support the potential of dragon's blood fruit to be a class I preservative agent. In addition, this is also reinforced by the potential of other rattan fruit species in the same genus. Several species in the genus Calamus, such as *Calamus travancoricus*, *Calamus manillensis*, *Calamus manan*, *Calamus ornatus* Bl, and *Calamus caesius* Bl have antioxidant activity, this was detected from the activity of triterpenoid compounds, phenolic and flavonoid compounds in the fruit that are able to counteract free radicals through scavenging activity on superoxide anions and hydroxyl

radicals in a concentration-dependent manner, inhibiting enzymes that play a role in ROS formation and blocking intracellular and extracellular oxidation[8,13]. In addition to antioxidant activity, some species in the Calamus genus also have antimicrobial activity against bacteria such as *Escherichia coli*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Propionibacterium acnes*, *Vibrio cholerae* and *Staphylococcus epidermidis*, with inhibition ranging from 8.5 mm to 13.33 mm [5]. The content of phytochemical compounds in fruits such as alkaloids is known to have potential antibacterial activity because it has an aromatic quartener group that is able to interact with DNA, can damage the integrity of the peptidoglycan component in the bacterial cell wall which will further inhibit the formation of the cell wall layer and trigger cell death [6]. The existence of biological activities in some Calamus genus is thought to also be possessed by dragon's blood fruit which strengthens its potential as a preservative agent to maintain oil conditions and improve the quality of VCO oil to be better and full of nutritional and health values.

CONCLUSION

The addition of dragon's blood fruit (*Calamus draco*) with a concentration of 40% into VCO has been proven to increase the total saturated fatty acids content by 97.28% and some unsaturated fatty acid profiles detected in this whole coconut VCO are palmitoleic acid, methyl linoleate, and methyl oleate. In addition, the addition of dragon's blood fruit with various concentrations is known to affect the peroxide number and iodine number as oil quality testing parameters. This can be seen from the 40% concentration of dragon blood fruit added, which can reduce the average peroxide number and increase the iodine number in VCO, thus indicating that the addition of dragon blood fruit can prevent free radical oxidation and the oil does not quickly experience rancidity. In addition, the high presence of lauric acid in each VCO sample, especially in the sample with the addition of dragon's blood fruit at 40%, can also strengthen the potential of dragon's blood fruit as a class I preservative agent that is able to prevent microorganism contamination and oil spoilage by indirectly preventing bacterial growth.

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