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Technique Mixed Enzymatic Virgin Coconut Oil And Ethanol Extract Of Lemongrass Improving Antioxidant And Ester Content

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Abstract

Lemongrass ethanol extract component as an effort to increase the antioxidant activity of enzymatic Virgin Coconut Oil (VCO). The purpose of this study was to analyze the antioxidant activity and its ester compounds in various concentrations of enzymatic VCO and ethanol extract of lemongrass. The analytical method to measure the antioxidant activity in the enzymatic VCO used DPPH and lauric acid by gas chromatography-mass spectrometry. The results showed that there was an increase in antioxidant capacity and esters in the VCO-ethanol extract of lemongrass compared to the control VCO. The antioxidant activity increased in a mixture of 10 ppm concentration obtained about 70% immersion percent while the detected esters were methyl laurate ranging from 8.30-16.98% and methyl linoleate about 2% against lemongrass compared to control VCO. This is interesting to develop because linoleate has potential as an antifungal compound.

Keywords: antioxidant, lemongrass ethanol extract, enzymatic, ester, VCO

Introduction

Virgin Coconut Oil (VCO) is a product made from coconut meat which has antiviral and antibacterial properties. VCO is able to support the immune system by freeing the body from harmful microorganisms because it contains ±53% lauric acid and about 7% caprylic acid. To maintain the quality of the VCO produced, the VCO in this study will be made enzymatically without using heating. Making VCO enzymatically using the help of protease enzymes derived from instant yeast as a catalyst in the fermentation process.

Virgin Coconut Oil (VCO) already has many beneficial ingredients for the body such as natural antioxidants and good levels of fatty acids. Leaf extract has the potential to contain antioxidants [1], according Reference [2] natural antioxidants as free antidote and [3] the high antioxidant activity of VCO added with methanol extract occurs because VCO contains a number of minor component extracts belonging to phenolic compounds that have antioxidant activity.

The selection of lemongrass in this study was also based on the high inhibitory power of lemongrass against free radicals. In the research of [4] stated that the antioxidant activity in essential oil in lemongrass stems is very high with an effective inhibition of 89 to 89.63%.

Based on the results of previous studies, in this study the manufacture of VCO will be carried out using an enzymatic method with the help of yeast to accelerate the fermentation process, and ethanol extract of lemongrass stems is added to increase the antioxidant capacity produced.

Materials and Method

Ingredients

Old coconut flesh, lemongrass stalks, instant yeast, 96% ethanol, water, aquadest, DPPH, and acetone.

Tool

Maceration vessel, mechanical stirrer (mixer), analytical balance, filter, grated coconut, cotton, rotary evaporator, centrifuge, vortex, beaker, measuring cup, funnel, dropper, UV-VIS spectrophotometer, GC-MS.

Procedure

1. Lemongrass Extract by Maceration

The lemongrass stalks that have been sorted are cut into small pieces and then dried. Drying is done by aerated for several days. The dried lemongrass stalks were then weighed as much as 500 g and put into a maceration container then added with 3 (three) liters of 96% ethanol solvent and left for 3x24 hours while stirring occasionally. The filtrate was collected and evaporated on a rotary evaporator to obtain a thick ethanolic extract. The extract obtained was weighed.

2. Making Enzymatic VCO Using the Stirring Method with the Addition of Ethanol Extract of Lemongrass Stem

Grated old coconut meat, then weighed as much as 1000 grams. Added 1500 mL of water and squeezed to produce coconut milk. The coconut milk is left for ± 4 hours to take the cream part. The coconut cream is put into a container, then stirred using a mechanical stirrer (mixer) for 20 minutes. 0.5 g of instant yeast is added to the coconut cream and stirred for 10 minutes. Incubation for 24 hours produces 3 layers, namely the top layer is VCO then followed by the blondo layer, and water. The formed VCO was then separated, and then added without and with ethanol extract of lemongrass stem which had been prepared with a concentration variation of 0; 5; 7,5; and 10% are VCO_0; VCO_5; VCO7,5; and VCO_{10} respectively.

3. Fatty Acid Profile Testing

A total of 100 µL of VCO sample was pipetted, put into a microtube and added 100 µL of methanol. The mixture was pipetted as much as 1 µL and then injected into the GC-MS tool.

4. Antioxidant Test

A sample of 0.2 g of enzymatic VCO was weighed and then dissolved with 5 mL of acetone, then vortexed and centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered to obtain a filtrate. This filtrate is diluted to a volume of 5 mL. The next step is to make several mixtures with the following ratio:

Mixture	DPPH (µL)	Sample VCO (µL)	Aseton (µL)
control	1000	-	-
Blank	-	-	500
Sample	1000	500	

The mixture was then incubated in the dark for 30 minutes and the absorbance value was read at 517 nm.

Result and Discussion

Based on the research conducted, 1000 g of grated coconut flesh was added to 1500 mL of water, resulting in 350 mL of coconut cream. In the incubation process, the container used to store coconut milk must really be tightly closed, so as not to be contaminated with air and cause bubbles to form in the coconut milk. 350 mL of coconut cream produced 105 mL of VCO (106.95 g), with a yield of 10.70%.

In this study, the production of enzymatic VCO was carried out before adding the enzyme using a mixer for 20 minutes to break the coconut milk emulsion. Coconut milk is an oil-in-water emulsion system, where the emulsifier that plays an important role in the system is protein. The stirring process is expected to destroy the stability of the lipoprotein so that eventually the oil and water can be separated. The stirring process is expected to increase the yield of VCO to be obtained.

The yeast Saccharomyces cerevisiae found in instant yeast produces proteolytic and amylolytic enzymes that are able to hydrolyze proteins into simpler peptide compounds. The breakdown of protein molecules causes it to no longer act as an emulsifier in coconut milk so that the oil and water will be separated.

The resulting VCO was then added with lemongrass ethanol extract with a concentration variation of 5%; 7,5%; 10% and without the addition of lemongrass ethanol extract as control.

Fatty Acids in Various VCO with GC-MS

Fatty acid testing was carried out on the four VCO samples, to determine the fatty acid composition in the VCO fatty acid analysis was carried out qualitatively and quantitatively using Gas Chromatography (GC) and Mass Spectrometer (GC-MS). The results of the analysis are in the form of a chromatogram which contains retention time, peak area, and concentration of each component of various VCO.

Table 1 below is the result of the comparison of the retention times produced in the GC-MS test on VCO with and without lemongrass ethanol extract. The results of this GC-MS test are in the form of retention time data, which has been compared with the standard retention times contained in the database, so that the types of fatty acids detected can be known.

Table 1. The retention time of methyl ester in control VCO and VCO-Lemongrass Ethanol Extract are diluted with methanol

Methyl ester	Retention time (minutes)			
	VCO_0	VCO_5	$VCO_{7,5}$	VCO_{10}
octanoate	4,528	4,522	4,528	4,522
decanoate	7,237	7,237	7,237	7,231
laurate	11,303	11,315	11,315	11,303
myristate	16,017	16,023	16,017	16,023
palmitate	18,882	18,882	18,882	18,882
linoleate	-	20,823	20,817	20,823

The VCO sample whose components were known by GC-MS analysis, then calculated the percentage of the components in the VCO. The results of the calculation of the relative percentage (%) of VCO components with and without the addition of lemongrass ethanol extract can be seen in Figure 1.

Figure 1. Percent Relative (%) methyl ester in VCO control and VCO with the Addition of Lemongrass Ethanol Extract

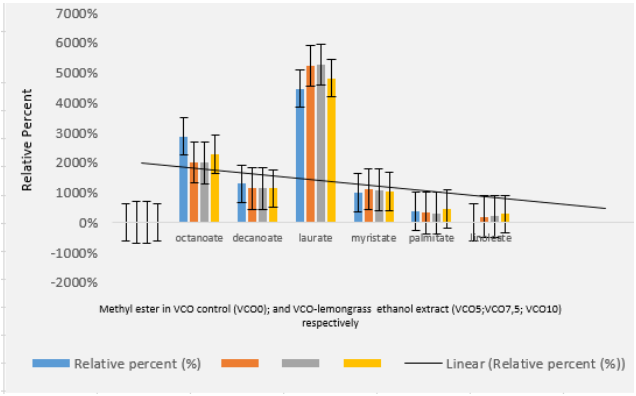

Figure 1 above shows that the largest component in VCO with and without the addition of lemongrass ethanol extract is lauric acid, which ranges from 44-52%. Natural antioxidants avoid rancidity [5] such as carrot powder [6] and fermented serai- saccharomyces cereviceae in VCO detected ester compound (m/z 88 and 74) by mass spectrometry [7] were also natural antioxidant in VCO and analyzed using GC-MS contains some esters such as ethyl laurate [8]. lauric acid about 50% of the total saturated fatty acids in VCO [9] . Lauric acid is a medium chain saturated fatty acid commonly called Medium Chain Fatty Acid (MCFA), which in the human body can act as anti-viral and anti-bacterial. Laurate was found to be less than 2 percent and palmitate about 20 percent in VCO-citronella evaporated with a rotary evaporator [10]. The combination of lauric acid and monolaurin was found to have higher bacterial counts ($p < 0.05$) than the combination of both lipid and lactic acid at sub-inhibitory concentrations [11].

The data in Table 2 shows an increase in the methyl linoleate content between VCO with the addition of lemongrass ethanol extract, and VCO without the addition of lemongrass ethanol extract. VCO with the addition of ethanol extract of lemongrass has methyl linoleate content ranging from 1-3%, while VCO that is not added with ethanol extract of lemongrass stems does not contain methyl linoleate. Methyl linoleate that appears in the VCO with the addition of ethanol extract of lemongrass stems may be caused by a reaction between the substances contained in the ethanol extract of lemongrass with the fatty acids in the VCO. According to SNI, VCO which can still be said to be of good quality contains 1-2.5% linoleic acid. Judging from the requirements set by SNI, it is known that VCO without the addition of lemongrass ethanol extract up to the addition of 7,5% lemongrass ethanol extract still meets the requirements for quality VCO. To prevent the oil from going rancid, the VCO with the lowest methyl linoleate content was chosen, namely VCO without the addition of ethanol extract of lemongrass stalks.

Antioxidant Activity in Various VCO

Antioxidants are substances that can reduce the negative effects of free radicals. Antioxidant activity in this study was tested by UV-VIS spectrophotometer with DPPH reagent. Based on the research conducted, the antioxidant activity was expressed as percent reduction (%), and the following results were obtained in Table 3.

Table 3 Percentage Reduction (%) Antioxidant VCO Control and With Addition of Lemongrass Ethanol Extract

Samples	Absorbance Sampel	Absorbance control	Percent attenuation (%)
VCO_0	0,1933	0,4045	52,21
VCO_5	0,1487	0,4045	63,24
$VCO_{7,5}$	0,1383	0,4045	65,81
VCO_{10}	0,1192	0,4045	70,53

Based on the data in Table 3 above, the smallest VCO antioxidant activity was VCO which was not added with lemongrass ethanol extract. VCO which was given the addition of lemongrass ethanol extract, experienced an increase in antioxidant activity, with the highest antioxidant activity in VCO with the addition of 10% lemongrass ethanol extract. This increase in antioxidant activity indicates that the hypothesis of adding a substance containing antioxidants to the VCO will increase the antioxidant activity of the VCO is correct. Based on the results of research by [3], VCO contains minor components in the form of phenolic compounds such as -tocopherol. Tocopherol acts as a scavenger of oxygen free radicals, lipid peroxy and singlet oxygen [12]. The high antioxidant activity in VCO with the addition of lemongrass ethanol extract is thought to be because the lemongrass stalks also contain extracts of minor components in the form of flavonoid compounds and tannins. According to [2] natural antioxidant compounds possessed by plants are generally phenolic compounds which can be in the form of flavonoid compounds, tocopherols and organic acids. The addition of ethanol extract of lemongrass causes the number of minor components contained, thereby increasing its antioxidant activity.

Antioxidants in oil can be interpreted as compounds that can delay, slow down and prevent lipid oxidation in coconut oil [5]. In addition to fatty acids, VCO is also known to contain several other chemical components such as sterols, vitamin E and the polyphenol fraction (phenolic acid). These chemical components have been reported to have antioxidant activity in various plant materials, food products and in biological systems.

Antioxidants can be analyzed by UV-Vis spectro- photometer due to the presence of DPPH reagent. The DPPH reagent has chromophore and auxocorm groups that can provide absorbance using a UV-Vis spectrophotometer. Testing of antioxidant activity was carried out by measuring the ability of VCO to capture DPPH radicals. This ability is characterized by a change in color from purple to yellow. The dark purple color of the DPPH solution is due to the unpaired electrons. Changes in the color of the solution to faded purple or turn yellow after the reaction is a positive result of the presence of antioxidants in the test sample.

The results of the calculation of the percent attenuation in Table 3, a curve is made with the variation of concentration as the x value and the percent attenuation as the y value. Based on the curve in Figure 2 that has been plotted, the equation of the line $y = 1.8058x + 0.5279$ is obtained which is then used to find the IC50 value. The IC50 value is the effective concentration of extract required to reduce 50% of the total DPPH.

Figure 2. Standard curve of Percentage variation of lemongrass ethanol extract in VCO with Immersion Percent

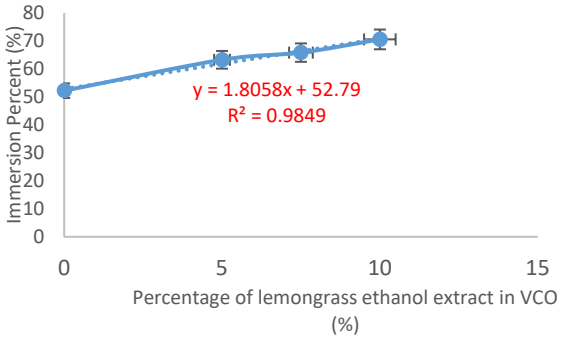

Figure 2 shows that the regression value obtained is 0.9849 where this value is close to 1, so it can be said that the relationship between concentration and absorbance is quite linear. The equation of the line y = 1.8058x + 0.5279 that has been obtained is used to calculate the IC50 value, by substituting the y value to be 50. Based on the calculation results in Appendix 3 point 7, the IC50 value is 27,396. According to [1], the smaller the IC50 value, the higher the antioxidant activity. If the antioxidant activity of an extract is below 50 ppm, the antioxidant activity is very strong. Based on the IC50 value which is less than 50 (27.396) the VCO samples studied can be categorized as strong antioxidants.

Conclusions and Suggestions

Based on the research that has been done, the authors can conclude that the effect of variations in the ethanol extract of lemongrass on the enzymatic Virgin Coconut Oil (VCO) produced is an increase in the antioxidant capacity of the enzymatic VCO along with the increase in the concentration of the ethanol extract of lemongrass. The lauric acid content of VCO with the addition of lemongrass ethanol extract which was analyzed by GC-MS was also higher when compared to VCO which was not given the addition of lemongrass ethanol extract. For further research, to pay attention to the type, age and growing area of the coconut to be used, in order to obtain consistent results in the repetition of making VCO.

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REFERENCES

P. Bahriul, N. Rahman, dan A.W. Diah, “UjiAktivitas Antioksidan Ekstrak Daun Salam (Syzygiumpolyanthum) dengan Menggunakan 1,1-Difenil-2-Pikrilhidrazil,” Jurnal Akademia Kimia. vol.3, no. 3, pp. 368-374, August 2014.

S. Kumalaningsih, “Antioksidan AlamiPenang kalRadikal Bebas, Sumber, Manfaat, Cara Penyediaan, dan Pengolahan,” Edisi ke-1. Trubus. Surabaya, 2006.

A. Muis, “Aktivitas Antioksid and an Antifotooksidan Komponen Minor dari Virgin Coconut Oil (VCO),” Jurnal RisetIndustri. Vol. 3, no. 2, pp. 86-93, Agustus 2009.

M.E.S. Mirghani, Y. Liyana, and J. Parveen, “Bioactivity Analysis of Lemongrass (Cymbopogancitratus) Essential Oil,” International Food Research Journal. Vol. 19, no. 2, pp. 569-575, 2012.

N.D. Siswati, Juni SU, danJunaini. “Pemanfaatan Antioksidan Alami Flavonoid Untuk Mencegah Ketengi kan Minyak Kelapa.” Jurusan Teknik Kimia. FTI UPN.

N.M. Suaniti, M. Manurung, O. Ratnayani, and A. A. I. S. J. Dewi, “The Quality of Coconut Oil Prepared Using Heating Technique with Addition of Carrot Powder (Daucuscarrota L) As Natural Antioxidant,” Jurnal Kimia (Journal of Chemistry). Vol. 13, no. 1, pp. 117-124, Januari 2019.

N.M. Suaniti, I W. B. Adnyana, M. Manurung, and D. A. R. C. Devi, “Study Mass Spectrometry from Virgin Coconut Oil-‘Serai Wangi’ (Cymbopogonnardus) by Fermented Using Saccharomyces cereviciae.” Proceedings of the 2nd International Conference on Biosciences and Medical Engineerings (ICBME2019). Bali, 11-12 April 2019.