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Research paper

Synthesis and Characterization of Cocozone Oil as Skin Care Ingredient

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Abstract

Cocozone Oil (CCO) is one of the ozonated oils, obtained by the ozonation process of Virgin Coconut Oil (VCO), that can be used as a material for skin care products. The purpose of this study was to determine: (1) the optimum time and ozone dose for CCO synthesis from VCO using ozonation; (2) the changes in physico-chemical properties of the oil; (3) the change in saturated-unsaturated fatty acids content and the existence any new substances in the CCO. The ozonation of VCO was carried out for 25 hours, with monitoring at the intervals of 4, 8, 12, 16, 20 and 25 hours, at a constant temperature (25 °C) with an ozone flow of 0.25 g/hr. From this study it can be concluded that: (1) The optimal time or dose of ozone required for the synthesis of CCO from VCO through the ozonation process was 25 hours or equivalent to 0.0208 gr O_3/ml VCO, (2) Physico-chemical characteristics of the CCO produced: resulting acid value (AV) was 2.71 mg/gram i.e. an increase of 630%; the peroxide value (PV) obtained was 238,77 mgrek/kg i.e. increase of 3,453 %; the Iodine value (IV) was 0 (zero) i.e. a decrease of 100%; and, the viscosity was 13.30 centipoice i.e. it rose 116%; (3) the total content of saturated fatty acid increased by 3.34% whereas the unsaturated fatty acid decreased by 98.83; and based on the analysis results of ^{13}C and ^{1}H NMR spectra, the resultant CCO contains a new substance, that is aldehydes.

Keywords: *Ozonation, virgin coconut oil (VCO), cocozone oil (CCO), Ozonated oil, physicochemical Characteristic.*

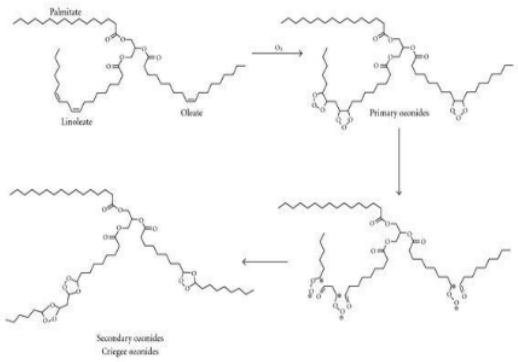
1. Introduction

Indonesia is a tropical country with diverse natural resources capable of producing vegetable oil, yet they are not fully utilized, for example, rice bran, cotton, palm, jatropha and coconut oil. Virgin coconut Oil (VCO) can be obtained from coconut milk using a mechanical process (quick stirring, pressing, filtration) and purification with Zeolite 3A as adsorbent (Handayani et al., 2016) without any heating process, so that the substances contained in the VCO are all natural. The fatty acids in VCO consist of 83.92% saturated fatty acids (C6:0 0.52%, C8:0 7.6%, C10:0 5.5%, C12:0 47.7%, C14:0 19.5% and C18:0 2.7%) and 7.8 % unsaturated fatty acids (C18:1 cis n-9 6.2 % and C18;2 cis n-6 1.6%) (Jana et al., 2015). VCO consists of 65% medium chain fatty acids (MCFAs) (Manisha et al., 2011) which are beneficial for health, especially for the skin because VCO is easily absorbed directly (Dayrit, 2003 and Dayrit, 2014).

The presence of unsaturated fatty acids in VCO of about 7.8% (Jana et al, 2015), enables the ozonation of VCO to produce cocozone oil with more active oxygen and other active substances simultaneously. Thus, the use of cocozone oil as a medicinal ingredient and for health can become more widespread. The oxidation reaction of unsaturated fatty acids by ozone to carbon double bonds occurs through the mechanism (Criegee, 1975; Bailey, 1978) as can be seen in Figure 1. (Travagli et al., 2010). The advantages of ozone gas (O_3) is that it is a strong oxidator, and is reactive and stable at low pH and temperature, so it easily reacts with unsaturated fatty acids or other unsaturated organic compounds (Langlai et al., 1991).

The compounds formed during the ozonation process of unsaturated fatty acids are 1,2,4-trioxolane or ozonide, aldehydes, carboxylic acids, peroxides, and poly peroxides, which are effective for skin tightening and wound healing due to infection by bacteria, viruses, fungi. and protozoa (Natalie et al., 2012; Zanardi et al., 2008; Diaz et al., 2005; Soriano et al., 2003). The study of ozonation in vegetable oils has been conducted by several researchers, mostly on olive oil, soybean oil (Sadowska et al., 2008), and sunflower seed oil (Diaz et al., 2006). The results are quite impressive and the resulting products have been sold in many countries as medicine for skin care.

The purpose of this study was to determine: (1) the optimum time and ozone dose for Cocozone Oil (CCO) synthesized from Virgin Coconut Oil (VCO) using ozonation; (2) the changes in physico-chemical properties of the oil; (3) the change in saturated-unsaturated fatty acids content and the existence any resultant new substances in the CCO.



Fig, 1. Reaction of Ozone with Unsaturated Fatty Acids via the Criegee Mechanism (Travagli et al., 2010)

2. Research Method

2.1. Solvent and Reagents

The Virgin Coconut Oil used in this study was derived from the original Indonesian coconut plant, produced with the brand name *Optima Indonesia*. Chemical reagents used were Chloroform (99%), concentrated Glacial Acetic acid, Potassium Iodide (99.5%), Ethanol (95%), Sodium Thiosulfate, Starch, Wijs solution, Phenol Phthalein and Sodium Hydroxide and Sulfuric acid (96%) from Merck.

2.2. The Ozonation Procedure

The instruments used for ozonation process were an ozone generator from West Technology Co., model CHS-212, voltage AC110V, and a cylindrical glass column reactor with an external jacket where the water flow is pumped from a thermostatic bath to maintain the temperature at the selected value. The dimension of the reactor was 800 mm and 40 mm ID, and it a diffuser inlet used to create bubbles in the ozone-oxygen gas mixture, a gas outlet, a sample port and a magnetic stirrer (Enjarlis et al., 2006).

The experiment was started by inserting the air-ozone mixture into the flask (KI solution) to determine the concentration of ozone in gaseous form. Ozone gas was produced by the ozone generator with a maximum ozone production capacity of 0,25 gr O_3/h. Next, 300 ml of VCO was ozonated in the reactor pipe at a temperature of 25°C for 4, 8, 12, 16, 20 and 25 hours. The CCO that was formed was then analyzed for its viscosity; acid, iodine and peroxide values; the content of saturated and unsaturated fatty acids; as well as the presence of new substances in the CCO.

2.3. Analysis of the Physical and Chemical Characteristic of the Oil

Measurements of oil viscosity before and after ozonation were from the Ostwald capillary viscosity at 25-30°C. The Peroxide value is the number that expresses, in miliequivalents of active oxygen, the quantity of peroxide contained in 1.000 g of the substance. Acid value is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the substance (Travagli et al., 2010). The Iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of the substance (Vinod et al., 2015). Nuclear Magnetic Resonance (NMR) spectroscopy was used to identify the oil compositions as a function of ozonation time (Sadowska et al., 2008). A Jeol Nuclear Magnetic Resonance operating at 500 MH was used. The ^{1}H NMR spectra were obtained at 1163 kHz spectral width with 45° pulse width; 40 scans and 21.23 kb of memory were used to obtain the spectra. The ^{13}C spectra were recorded at 220 ppm spectral width with a relaxation delay of 2 s; a total of 491 scans were collected for each sample with a 30° excitation pulse.

3. Result and Discussion

3.1. Physico-Chemical Characteristics in VCO

Table 1 is the change of the values (acid, peroxide and iodine) and viscosity of the VCO (before ozonation) and Cocozone Oil (after ozonation). From the table it can be seen that the peroxide and acid values, as well as the viscosity, increase with increasing ozonation time, while the iodine value decreases to zero. After oil ozonation for 25 hours (equivalent to 0.0208 gr O_3/ml VCO), the peroxide value obtained was 238.77 meq/kg i.e an increase of 3,453.13%, the acid value 2.71 mg KOH/g i.e. an increase of 630%, the viscosity 13.30 Centipoice i.e. an increase of 116%, and the iodine value reached 0 (zero) i.e. a decrease of 100%, compared to the oil before ozonation. This is due to the oxidation of unsaturated fatty acids in the oil by ozone through the Criegee mechanism, such as the reaction in Fig. 1, and it produces active oxygen, peroxides and free fatty acids. Increased ozone time causes the amount of ozone in the oil to rise; consequently, unsaturated triglycerides and unsaturated fatty acids in the oil are increasingly oxidized by the ozone. This heightens the content of saturated fatty acids, free fatty acids, and active oxygen or peroxide, which is indicated by the cocozone oil iodine value reaching zero, the acid and peroxide values increasing significantly (Sandowska et al., 2008) and, visually, the cocozone oil being more viscous than the VCO.

The peroxide value of cocozone oil obtained (238.77 meq / kg) was much smaller than that of olives and sunflower ozonized respectively of 2439 and 2506 meq / kg. Thus Cocozone Oil is more suitable and safe to use as an agent to maintain health and care for the skin such as cleaning makeup, moisturize the skin, tighten skin wrinkles, massage, cleans and sterilizes the epidermis, diaper rash and mild infection of the skin (Almeida et al, 2012).

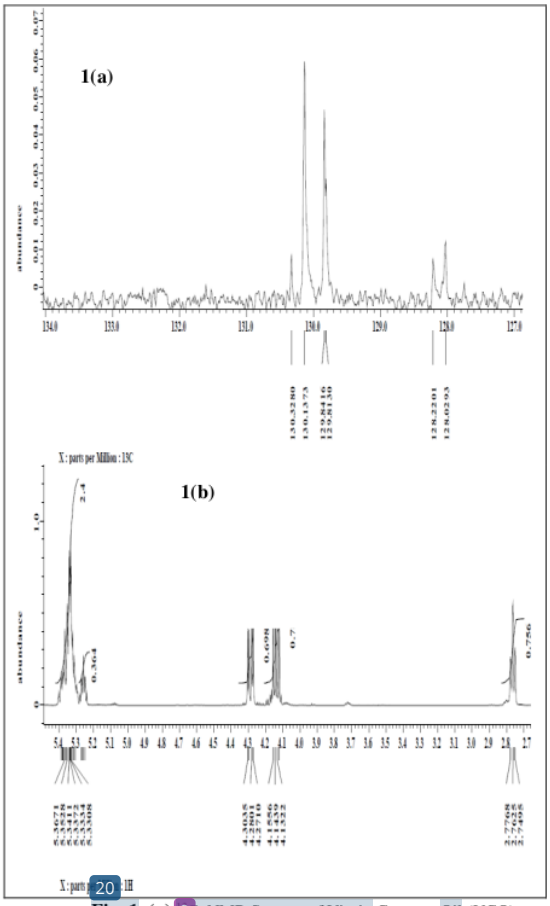
Table 1. Physico-Chemical Characteristics of VCO and CCO

Oil	t (hr)	AV (mgKOH/g Oil)	PV (meq/ Kg Oil)	IV (grI_2/ Kg Oil)	Viscosity (cp)	Ozone Contents (g O_3/ ml Oil)
VCO	**0**	**0.37**	**6.72**	**8.02**	**6.16**	**0.00**
CCO	4	0.59	34.82	7.98	8.07	0.0033
CCO	8	0.73	64.18	6.62	9.10	0.0067
CCO	12	1.12	94.96	5.27	10.97	0.0100
CCO	16	1.76	150.13	3.03	11.85	0.0133
CCO	20	1.96	186.63	2.36	12.30	0.0167
CCO	**25**	**2.71**	**238.77**	**0.00**	**13.30**	**0.0208**

3.2. Change of Functional Groups

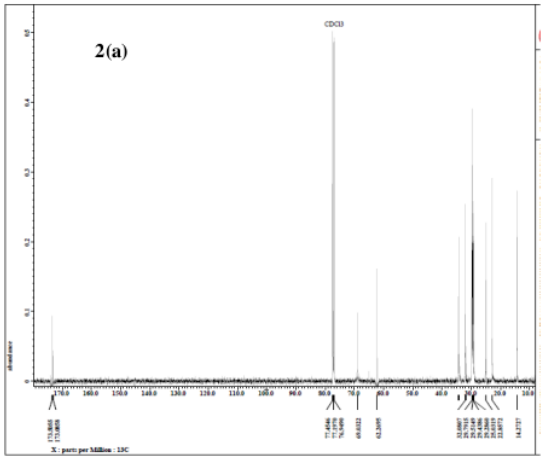
VCO contains unsaturated fatty acids, such as linoleic acid (C18:2) and oleic acid (C18:1) (Yong et al., 2009). ^{13}C and ^{1}H NMR spectra were used to determine the carbon alkenes peak; Figures 1(a) and 1(b) show the spectrums of ^{13}C and ^{1}H isotopes in VCO. From the literature, it is known that the unsaturated fatty acids have unique spectrums at 128-130 ppm in the ^{13}C spectra and ^{1}H NMR spectra tagged the photon spectrum from 4.9 to 5.9 ppm (Sandowska et al., 2008 and Travagli et al., 2010). Oleic acid has one double bond and is represented by two distinct peaks on the spectrum of 130 ppm in the ^{13}C-NMR analysis. Linoleic acid has two separate double bonds and two additional distinctive peaks in the 128 ppm spectrum with ^{13}C-NMR analysis (Sega et

al., 2010). The ^{13}C NMR test for VCO also proves that there was a C=O group at the spectrum of 173 ppm, CH_2 at the spectrum of 14-35 ppm, C-H_3 at the spectrum of 14 ppm, and the OH group in the 60-70 ppm spectrum. Linoleic and oleic acid contained in VCO reacted to ozone to form trioxolane, aldehyde and hydroperoxide (Diaz et al., 2005; Jardines et al., 2003).



Fig, 1. (a) ^{13}C NMR Spectra of Virgin Coconut Oil (VCO)
1. (b) ^{1}H NMR Spectra of Virgin Coconut Oil (VCO)

Figure 2(a) shows the ^{13}C-NMR spectra of the cocozone oil (ozonated oil). The figure does not show the signal C=C. This is because C=C in the unsaturated fatty acid has reacted with ozone to form trioxolane which quickly turned into aldehydes and peroxides (Sandowska et al., 2008). ^{1}H NMR spectra measurements of ozonated oil found the proton spectrum of CH_3 at 0.8 to 0.9 ppm, the proton spectrum of CH_2 at 1.2 to 1.6 ppm, the proton spectrum of CH_2 at 2.3 ppm and tangent to C carbonyl group. 4.1 to 4.3 ppm is still a proton signal of C=C and 5.2 ppm is also for proton spectrum C = C and at 9.7 ppm (9.4 to 10.4 ppm) for proton spectrum aldehyde group (Bailey, 1978; Sandowska et al., 2008).



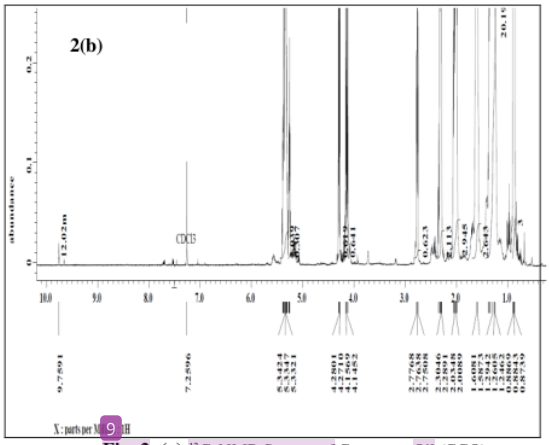


Fig. 2. (a) ^{13}C NMR Spectra of Cocozone Oil (CCO)
2.(b) ^{1}H NMR Spectra of Cocozone Oil (CCO)

3.3. Fatty Acid Composition in VCO and CCO

The effect of ozonation on virgin coconut oil, specifically on the fatty acid content in VCO, was identified using GC-MS. VCO is the raw material in the production of CCO, containing saturated and unsaturated fatty acids. Table 2 lists the composition of fatty acids in VCO and CCO. From the table, it can be seen that the percentage of unsaturated fatty acids (oleic) in VCO goes down from 5.39% to 0.07% and linoleic acids go down from 1.9% to 0% after ozonation (in CCO). The total content of unsaturated fatty acid decreased by 3.34% while as the unsaturated fatty acid increased by 98.83. This is the result of the reaction between ozone and unsaturated fatty acids using the Criegee method. The reaction of ozone with virgin coconut oil occurred almost exclusively in the carbon-carbon double bonds contained in the unsaturated fatty acids (Oleic and linoleic) (Bailey, 1978).

Table 2: Saturated and Unsaturated Fatty Acid Composition in Oil (VCO and CCO)

Fatty Acid	VCO (%)	CCO (%)	Increase or Decrease (%)
Saturated Acid			Increase
Caprilat (C8)	5.78	8.13	
Caprate (C10)	7.42	7.42	
Lauric (C12)	51.30	51.30	
Miristic (C14)	16.60	17.30	
Palmitate (C16-0)	10.10	10.10	
Stearate (C18-0)	2.84	2.94	
Total	**94.04**	**97.18**	**3.34**
Unsaturated Acid			Decrease
Oleic (C18-1)	5.39	0.07	
Linoleic (C18-2)	0.59	0.00	
Linolenat (C18-3)	0.00	0.00	
Total	**5.98**	**0.07**	**98.83**

3. Conclutions

From this study it can be concluded that: (1) The optimal time or dose of ozone required for the synthesis of Cocozone Oil from VCO through the ozonation process is 25 hours or equivalent to 0.0208 gram O_3/ml VCO, (2) For the physico-chemical characteristics of the Cocozone Oil produced: the resulting acid value was 2.71 mg/gram i.e. an increase of 630%; the peroxide value obtained was 238,77 mgrek/kg i.e. increase of 3,453 %; the Iodine value was 0 (zero) i.e. a decrease of 100%; and, the viscosity was 13.30 centipoice i.e. it rose 116%; (3) In the Cocozone Oil produced, the total content of saturated fatty acid increased by 3.34% whereas the unsaturated fatty acid decreased by 98,83%; also, the ^{13}C and ^{1}H NMR analysis identified the presence of aldehyde compounds.

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