

Effect of pH and Ozone Dosage on Characteristic of Ozonated Rice Bran Oil

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ABSTRACT. The influence of pH and ozone dose, as well as ascorbic acid addition during the ozonation process, on the properties of Rice Bran Oil (RBO), was examined. The spectroscopic characteristic of RBO before and after ozonation was analysis directly, while the physicochemical property was assessed by density, viscosity, pH, iodine number, peroxide number, and acid number. With an increase in ozone dose, the carbon double bond in the RBO reduced. The primary product of the ozonation process is ozonide, and one of its by-products is 1,2,4-trioxolane, which contains a carbon single bond as a result of the ozonation reaction. According to this study, the pH 4 and ozone dose of 440 mg O₃/L are the optimum parameters utilized in the RBO ozonation process. RBO's density and viscosity were 0.918 g/mL and 0.042 cP, respectively, at these conditions. Its iodine number, acid number, and peroxide number were also 3.173 g/g RBO, 2.3 mg NaOH/g RBO, and 55 mg_{eq}/kg, respectively. Analyses of gas chromatography and nuclear magnetic resonance spectroscopy revealed the presence of 1,2,4-trioxolane. Ozone dosage is critical because greater ozone concentrations place RBO in a saturated state, making the 1,2,4-trioxolane unstable and readily destroyed, whereas lower temperatures can avoid this.

Keywords: vegetable oil; ozonation; additive; trioxolane; peroxide number

INTRODUCTION

Rice bran is a by-product of rice milling that is mostly used as animal feed at the moment. Rice milling generated 20% rice husk, 8% rice bran, and 2% rice germ (Van Hoed et al., 2006). Rice bran is formed from the rice grain's outermost layer, which is located between the rice grains and the rice bran. It contains necessary nutrients such as protein, fat, carbohydrates, and calories (Saleh et al., 2019). Rice bran oil (RBO) may be generated from rice bran and contains both saturated and unsaturated fatty acids in the form of palmitic acid and oleic and linoleic acids (Orthoefer, 2005). Historically, the rice bran waste processing output has lacked commercial value. For instance, consider its use as animal feed (Sharif et al., 2014).

Due to the high concentration of unsaturated fatty acids in RBO, such as oleic and linoleic, it may be used as a raw material for the creation of trioxolane and peroxide as an active medicinal component through the Ozonation process with Criegee mechanism (Criegee, 1975), which is beneficial in the body's fight

against free radicals, as shown in **Figure 1**. Guerra-Blanco et al. (2021) created ozonated vegetable oil from a variety of vegetable oils and investigated their kinetic response; moreover, the research discovered that the density and viscosity of the ozonated vegetable oil varied significantly. Previous study from RBO's NMR test suggested that the 1,2,4-trioxolane group was still searching for and obtaining only its derivative compounds, mainly aldehydes and peroxides (Enjarlis et al., 2019).

According to prior research, trioxolane was not detected in the RBO's NMR test, although peroxide was. This might be due to unstable ozone being created and failing to participate in the reaction as a result of an incorrect analytical reaction. Thus, additional research is needed on the synthesis of ozonated oil from RBO using the proper technique and the addition of acid additives such as ascorbic acid to stabilize the ozone. We claim this as the academic novelty of our study. As ozone is stable at acidic pH, the addition of ascorbic acid attempts to reduce the pH of the RBO

and ascorbate emulsion combination until it achieves a low pH. According to Hoigne et al. (1985) at high pH levels, hydroxyl ions cause ozone to breakdown into H-O_2 and O_2 , which subsequently transform into hydroxide radicals ($\cdot\text{OH}$). According to Buffle et al. (2006), pH 2 ozone breakdown is slow (stable), and as pH rises, so does ozone decomposition. According to the Criegee mechanism (Criegee, 1975), trioxolane and peroxide are produced when the double C bonds in unsaturated fatty acids (oleic, linoleate) react with ozone. It is hoped that trioxolane, peroxide, and other novel chemicals generated during the ozonation of RBO will be discovered.

EXPERIMENTAL SECTION

Sample Preparation and Reaction Configuration

The RBO sample used is commercial grade with brand of Oryza Grace from Kasisuri Co. Ltd. (Ayutthaya, Thailand), which is often found in supermarket. The characteristic of RBO can be shown in **Table 1**. Each sample requires roughly 4 L of RBO. Then RBO is mixed with ascorbic acid concentration 1,0 M with ratio of Ascorbic acid to RBO 0.092 for pH 2.0, 0.06 for pH 3.0 and 0.03 for pH 4.0, which acts as an additive and acidity controller. Solid ascorbic acid (Merck, New Jersey, United States) is dissolved in distilled water until saturated. The composition of 100 mL of ascorbic acid solution was 82.398 mL of water and 17.612 g of ascorbate. The saturated ascorbic

acid was then added to the RBO sample, which had a pH of 6. Adding ascorbic acid until the pH of the RBO reached 2, 3, and 4. The ozonated RBO was synthesized using a technique reported by Zanardi et al. (2008). The RBO sample was then ozonated in a glass ozone reactor that was put into a small aquarium filled with cold water that was constantly pumped from another aquarium filled with water and ice cubes. Previous investigations have found that the good conditions for the ozonation process are at a temperature of 5 °C (Elovitz et al., 2000). A magnetic stirrer is also installed in the glass reactor to swirl the oil and optimize the mixing of oil with ozone. X-troy CHS-212 ozone generator from Taizhou Shengjie Air Purifier Co., Ltd. (Zhejiang, China) used as ozone generator. Because the input gas for the ozone generator originates from the medical oxygen cylinder, the input gas for the ozone generator is pure oxygen gas (98-99%) with the gas flow were 0.1075 mg/mL.h. The ozone output from the generator is pumped into the oil in a glass reactor via a silicon pipe, and a diffuser is fitted to improve ozone absorption into the oil. RBO was ozonated by varying the ozone dose; 150, 210, 270, 330, 380, and 440 gr of O_3 /L with reaction time were 84, 117, 151, 184, 212, and 246 min, respectively. The ozonated RBO was then kept at 10 °C before being utilized for analysis and characterization.

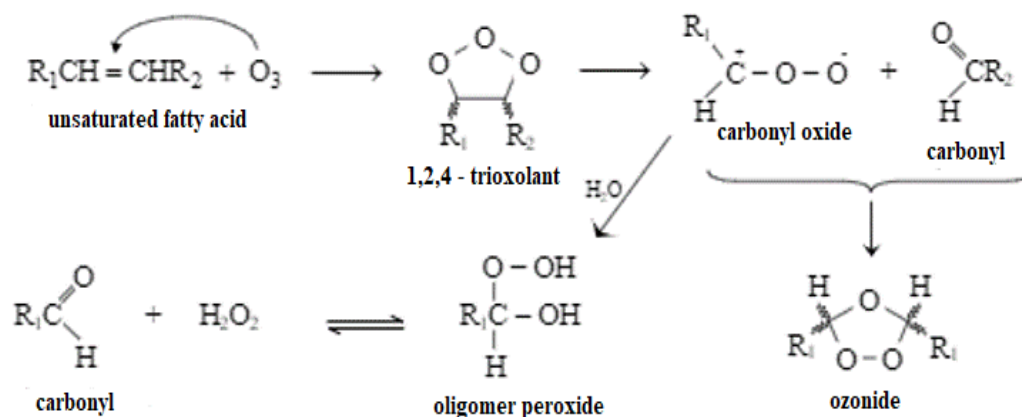


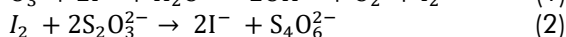
Figure 1. Ozonation mechanism in unsaturated fatty acid

Table 1. Characteristic of RBO before ozonation process

Composition	Value	Unit
Iodine Number	147.204	g Iodine/100 g Oil
Peroxide Number	5	mg _{eq} /kg Oil
Acid Number	1.3	mg NaOH/g Oil
Viscosity	0.034	cP
Density	0.833	g/mL
pH	5	
Energy	802	kcal/100 mL Oil
Total Fat	90	mg/100 mL Oil
Gamma Oryzanol	229	mg/100 mL Oil
Vitamin E	7	mg/100 mL Oil

Iodine Number Analysis

In this experiment, iodometric titration was performed to quantify the quantity of ozone consumed by the oils/fatty acids. As indicated in Equation 1, the reaction between iodide and ozone produced free iodine. Then, as indicated in Equation 2, iodine was reacted with sodium thiosulphate (Merck, New Jersey, United States), and starch (Merck, New Jersey, United States) served as an indicator. The hue would shift from purple to colorless as an indicator of equivalency point (Sadowska et al., 2008).



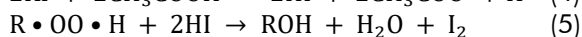
De-Ionized (DI) water served as a control solution. The Iodine Number (IN) was determined using Equation 3:

$$\text{IN} = \frac{(V_{\text{blank}} - V_{\text{sample}}) \times N_{\text{titrant}} \times \text{mEq I}_2}{m_{\text{sample}}} \quad (3)$$

where $m_{\text{eq I}_2}$ is the weight equivalent of iodine; V_{blank} and V_{sample} are the titrant volumes used to titrate the blank and sample solutions until the equivalence point, respectively. N_{titrant} denotes the titrant's normality, and m_{sample} denotes the mass of RBO.

Peroxide Number Analysis

Peroxide Number (PN) is the number that represents the amount of peroxide, in milli equivalents of active oxygen, that is contained in 1000 g of the material, according to British Pharmacopoeia (2000a). The PN of both untreated and ozonated samples was measured using the approved technique of the American Oil Chemists' Society (AOCS), utilizing the reaction process described in Equations (5) and (6):



In the presence of acetic acid (Smart Lab, South Tangerang, Indonesia), peroxide ($\text{R} \cdot \text{OO} \cdot \text{H}$) will react with potassium iodide (Merck, New Jersey, United States) to produce iodine. The iodine is then titrated with a sodium thiosulfate solution as described in Equation (2). The PN is determined using the formula in Equation (7) (AOCS, 1998):

$$\text{PN} = \frac{V \times c_{\text{titrant}} \times 1000}{m_{\text{sample}}} \quad (6)$$

where V is the volume of the titrant $\text{Na}_2\text{S}_2\text{O}_3$; c is the concentration of the $\text{Na}_2\text{S}_2\text{O}_3$; and m_{sample} is the mass of the RBO.

Acid Number Analysis

According to the British Pharmacopoeia (2000b), the Acid Number (AN) is the number of base mass necessary (in mg) to neutralize the free acids per gram of the material. Furthermore, the acid value indicates how much the triglycerides in the oil sample have broken down to create free fatty acids. To titrate the mixed solution of the oil sample and ethanol (Smart Lab, South Tangerang, Indonesia), sodium hydroxide (Kanto Chemical, Tokyo, Japan) was employed as a titrant, and phenolphthalein was utilized as an indicator (de Almeida Kogawa et al., 2004). Equation (8) was used to determine the AN:

$$\text{AN} = \frac{\text{MW}_{\text{titrant}} \times c_{\text{titrant}} \times V_{\text{titrant}} \times f}{m_{\text{sample}}} \quad (7)$$

where MW denotes the molecular weight of NaOH as the titrant; c_{titrant} denotes the concentration of NaOH; and V_{titrant} denotes the volume of NaOH utilized. The mass of the oil sample is given by m_{sample} , while the correction factor is given by f .

Density, Kinematic Viscosity, and pH Analysis

The density was determined by weighing a pycnometer in the absence and presence of RBO samples. At 25 °C, kinematic viscosity and pH were determined with Ostwald capillary viscometers and a digital pH meter, respectively.

NMR, HPLC, and GCMS Analysis

The NMR spectra on untreated and ozonated RBO were obtained using a JEOL JNMEX400 single pulse spectrometer (Seoul, South Korea) at 25 °C. All of the tests were carried out under identical experimental settings and concentrations. The spectra were obtained with a relaxation delay of 2 s and a total of 1021 scans for each sample using a 30-excitation pulse.

Gas Chromatography Spectrometry (GCMS) GC Agilent 7890B tandem MSD 5977 A (California, USA) using n-Hexane as a solvent was used to evaluate the fatty acid. The sample to be examined was taken $\pm 10 \mu\text{L}$, then mixed into n-Hexane solvent up to 1000 mL, then swirled and sonicated for 15 minutes until completely dissolved. Derivation reagent $50 \mu\text{L}$ was added to the mixture, then heated in an oven at 60 - 70 °C for 30 minutes. Samples that have been prepared are ready to be injected into GCMS.

For the analysis of vitamins C and E in RBO before and after ozonation, a Waters Alliance 2695 HPLC System with 2489 Dual Absorbance Detector (California, USA) is employed. By dissolving vitamin C or vitamin E solution in aquadest, a standard solution with a concentration between 0.05 and 0.2 ppm can be created. The standard solution is then injected (from a low concentration to a high concentration), followed by the injection of the sample solution.

RESULTS AND DISCUSSION

Effect of pH and Ozone Dosage on Viscosity of Ozonated RBO

The effect of pH of the ozonation process on viscosity is shown in **Figure 2**, where the ozone dose is 440 mg O_3/L , and with lower pH, the value of viscosity and density rises. The greatest viscosity and density values were observed at of variable pH 2 with an ozone dosage of 440 mg O_3/L . When compared to Blank, which has a pH of 6, the acidic RBO has a higher viscosity. The viscosity of triglyceride fats increases as the unsaturated chain length decreases (Sadowska et al., 2008). Because of the poor water solubility in oil, when water is introduced during the ozonation process, the viscosity increases owing to the development of an emulsion (de Almeida Kogawa et al., 2004).

Effect of pH and Ozone Dosage on Density of Ozonated RBO

The density value increases in direct proportion to the rise in viscosity, therefore the higher the viscosity of the ozonated oil, the higher the density. The more ozone doses utilized in the RBO ozonation process, the newer chemical compounds generated, particularly peroxide and aldehyde compounds (**Figure 3**), causing the density of the oil to rise. Because ascorbic acid is more soluble in oil at pH 2, the density is lower at pH 4 (de Almeida Kogawa et al., 2004). In comparison to a blank with a pH of 6, the more acidic the RBO, the higher the viscosity. Another study on the ozonation of vegetable oils discovered that the drop in ester chain levels was caused by a decrease in unsaturated fatty acids owing to ozonation, which resulted in the production of new chemical compounds with a higher molecular mass, specifically the formation of oligomers (de Almeida Kogawa et al., 2004).

Effect of pH and Ozone Dosage on Acid Number of Ozonated RBO

Figure 4 depicts the influence of dosage and pH of the RBO ozonation process on the acid number. According to the graph, the highest acid number value is observed with an ozone dosage of 440 mg O₃/L and a pH of 2. This is because of the huge number of ozone doses that react with more and more unsaturated fatty acids, increasing the value of the acid number. The rise in acid number is due to the following factors: (1) Ozone is stable at acidic pH (pH 4), resulting in direct ozonation by O₃ (Pera-Titus et al., Giménez & Esplugas, 2004; Langlais et al., 1991), (2) The amount of ozone reacting with unsaturated fatty acids produces more ozonide/trioxolane, because trioxolane is unstable and easily converts to carboxylic acid and other products; and (3) the addition of ascorbic acid solution to the RBO to lower the pH automatically increases the volume of water in the RBO. As a result, the acid number will rise during

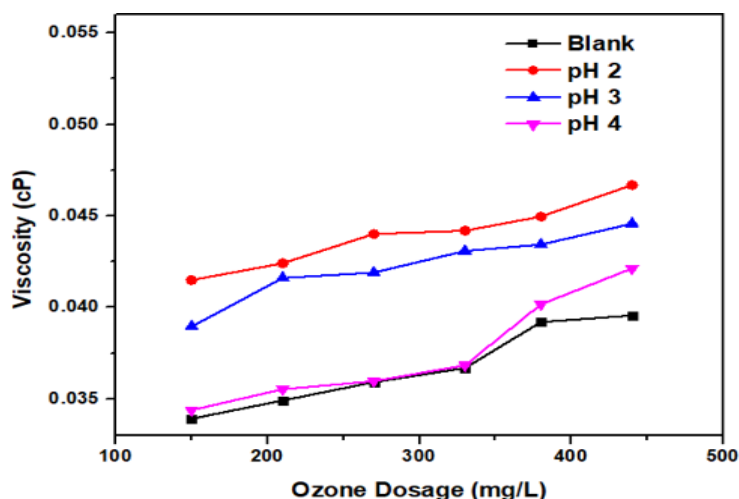


Figure 2. The Effect of pH and Ozone Dosage on Viscosity of Ozonated RBO

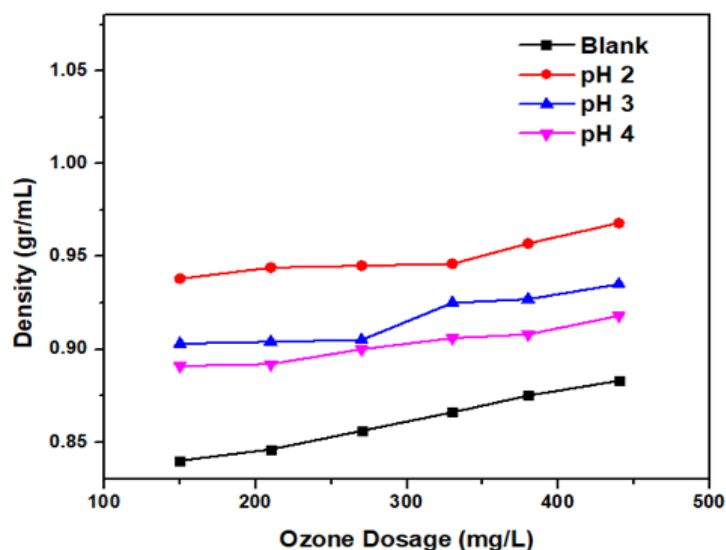


Figure 3. The Effect of pH and Ozone Dosage on Density of Ozonated RBO

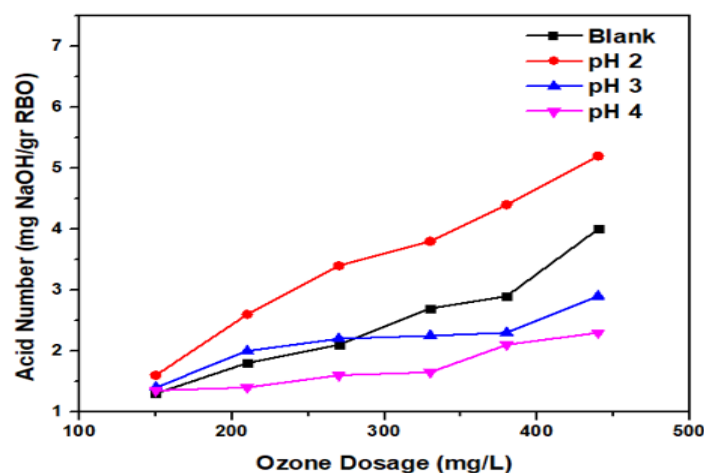


Figure 4. The Effect of pH and Ozone Dosage on Acid Number of Ozonated RBO

ozonation (de Almeida Kogawa et al., 2004; Wulansarie et al., 2019). The rise in acid number also indicates the product's acidity level and an index of degradation by-products or a sign of an increase in the breakdown process of unsaturated fatty acids (Travagli et al., 2010; Moulydia et al., 2018).

Effect of pH and Ozone Dosage on Peroxide Number of Ozonated RBO

Figure 5 depicts the influence of dosage and pH on the RBO ozonation process on peroxide number. The higher the ozone exposure, the higher the peroxide value. The greatest peroxide value was obtained at pH 3 with a dosage of ozone of 440 mg O_3/L and a peroxide value of 70 mg_{eq}/kg , which rose by 1300 % over the blank peroxide value of 5 mg_{eq}/kg . RBO oil conducts a redox reaction process at the peroxide value, with the redox reaction originating from two ideas, namely reduction and oxidation. When unsaturated oil is ozonated, molecules with double bonds undergo reduction, which opens the double bonds and allows ozone compounds to enter and replace the double bonds. The rise in peroxide value is caused by the high dosage of ozone, which interacts with unsaturated fatty acids and raises the

peroxide number. Ascorbic acid (vitamin C), a lactone (ester-in hydroxycarboxylic acid) with an enediol group as a strong reducing agent, caused the greatest increase in peroxide value at pH 4 (Naidu, 2003). The addition of ascorbate solution was less at pH 4 than at pH 3 and pH 2, indicating that RBO contains fewer strong reducing agents at pH 4, allowing the oxidation process by ozone to run more optimally at pH 4, allowing ozone to oxidize the double bonds of oil more easily because they are not blocked by the reducing group of the ascorbate solution. According to the explanation above, the rise in the maximum peroxide value shows that the ozonation process at high ozone doses causes numerous double bonds in the oil that are oxidized by ozone to enter with larger concentrations. Peroxides are formed as a result of the Criegee Mechanism, which shows indications of ozone breaking the double bonds of unsaturated fatty acids in oil (Balchum et al., 1971). Furthermore, the peroxide number is a value that may be used to measure the degree of damage and oxidized characteristics of the oil, as well as to evaluate the stability of the ozonated vegetable oil as a standard value for the oil's commercialization (Travagli et al., 2010).

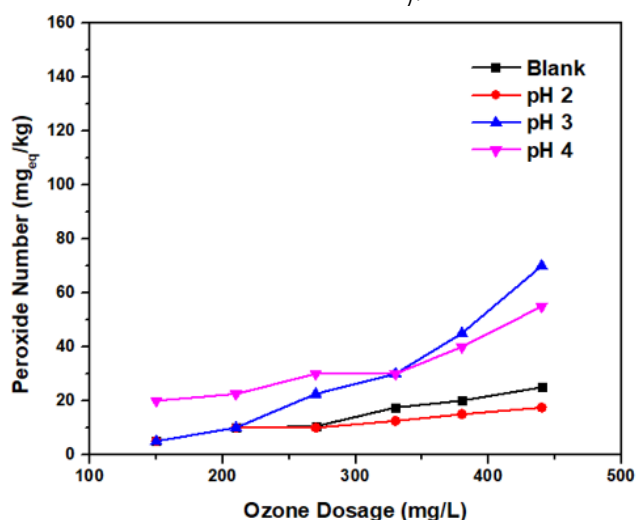


Figure 5. The Effect of pH and Ozone Dosage on Peroxide Number of Ozonated RBO

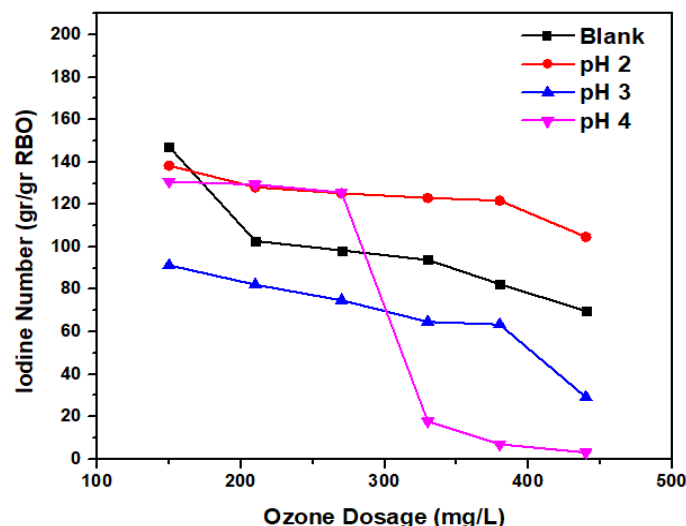


Figure 6. The Effect of pH and Ozone Dosage on Iodine Number of Ozonated RBO

Effect of pH and Ozone Dosage on Peroxide Number of Ozonated RBO

Figure 6 shows that the pH 4 variable and the ozone dosage of 440 mg O_3/L had the largest percentage drop in the value of the iodine value, with values of 97.84 %. These findings show an increase in the percentage decline in iodine. These findings suggest that the higher the ozone dosage used in the ozonation of oil, the lower the iodine number. The pH level reveals that pH 4 has the lowest iodine number. A reduction in the iodine number indicates the breaking of the double bond owing to the breakdown by ozone generating single bonds in unsaturated fatty acids that create saturated compounds due to the Criegee mechanism. The higher the ozone dosage in the RBO ozonation process, the lower the value of the RBO iodine number because more ozone breaks the double bond (de Almeida Kogawa et al., 2004).

The reaction mechanism for the oxidation of RBO unsaturated fatty acids by ozone is that there are three types of unsaturated fatty acids, namely oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). The graph indicates that the drop in iodine number is greater at pH 4 than at pH 3. This demonstrates that the lower the concentration of ascorbic acid in the RBO, the lower the iodine number. The quantity of ascorbic acid solution in RBO is smaller at pH 4 than at pH 3. At pH 4, the oxidation process of unsaturated fatty acids by ozone is enhanced because RBO contains less enediol groups (strong reducing agents) (Naidu., 2003) and at pH 4-6 Ascorbate is more stable (Moser U and Bendich A.,1990), which allows ozone to break double bond and run more efficiently As more unsaturated fatty acids are broken double bonds by ozone, the findings read in the iodine number test become lower as the number of double bonds decreases. Because of the oil's high level of unsaturation, it is more quickly oxidized (Zanardi et al., 2008).

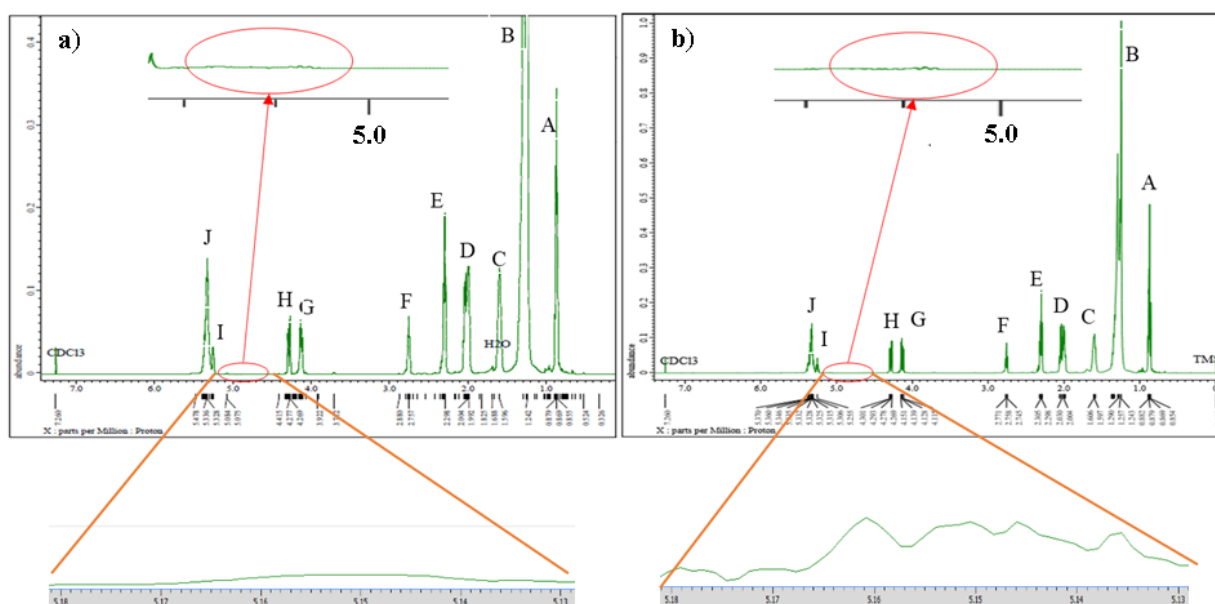
1H and ^{13}C NMR Analysis

Ozonated RBO oil samples examined in 1H and ^{13}C NMR were RBO with pH 4 adjustments and ozone dosage of 440 mg O_3/L since the results acquired the best parameter. The reaction that happens throughout the ozonation process is an ozone addition reaction to the double bond in unsaturated fatty acids. The double bond in unsaturated fatty acids was broken and replaced with an O bond to produce a new ozonide molecule (1,2,4-trioxolane). Figure 7a shows 10 different proton signals from the RBO sample without ozonation that there are 10 kinds of protons (H) from these compounds. The position of the proton signal is also different because it has a different intensity and area of chemical shift. The next step will be done by looking at the integral value of each signal will be marked with the letters A, B, C, D, E, F, G, H, I to make it easier to analyze as shown in Figure 6b. Figures 7a and 7b provide signal, chemical shift, number of protons, proton type, and compound type information. The NMR data of RBO non-ozonated can be seen in Table 2.

The chemical shift (1.2 ppm – 3 ppm) indicates the presence of CH_2 compounds, with (B) = 1.3 ppm indicating compounds with CH_2 that are not bound to the carbonyl, as indicated by the presence of multiples of the formation of protons ozonide, (C) = 1.67 ppm (CH_2CH_2COOH), (D) & (E) = 2.03 ppm & 2.3 ppm indicating CH_2 which is the presence of glycerol and triglyceride molecules is indicated by triplets at (G) and (H) = 4.1 – 4.35 ppm (CH_2OCOR). It indicates the presumptive triplet at (F) = 5.34 ppm with the triplet highlighted by the proton olein signal. The presence of high amounts of unsaturated fatty acids present in the RBO is indicated by (F) = 5.34 ppm with the triplet characterized by the proton olein signal, with the top peak indicating oleic acid and the second peak showing linoleic acid. At (I) = 5.29 ppm, chemical molecules with double bonds are found in greater concentrations in RBO than in other unsaturated fatty

Table 2. ^1H NMR and ^{13}C NMR assignments of RBO non-ozonated

δ_{C} [ppm]	δ_{H} [ppm]	Functional group	Assignments
172.9-173.4		Carboxylic acid	
128.2	5.34	$\text{CH}=\text{CH}$	All unsaturated fatty acids
130.1	5.29	$\text{CH}-\text{OCOR}$	Triglycerides
62.3; 69.0	4.16; 4.36	CH_2-OCOR	Triglycerides
24.9	2.78	$\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}$	Linolenic and linoleic chains
33.19	2.31	CH_2-COOH	All acyl chains
26.5	2.03	$\text{CH}_2\text{CH}=\text{CH}$	All unsaturated acyl chains
22.1	1.67	$\text{CH}_2-\text{CH}_2\text{COOH}$	All acyl chains
29–31	1.25–1.5	$(\text{CH}_2)_n$	All acyl chains
14.2	0.82	CH_3	Methyl group

**Figure 7.** The ^1H NMR spectra for RBO a) before and b) after ozonation process. Inset shows the ozonide ^1H NMR spectra

acids with double bonds. There is a novel chemical shift in the Ozonated oil spectrum with a range of 5.13 to 5.18 ppm in the form of a single sloping triplet signal, which refers to 1,2,4-trioxolane. The signal is a 1,2,4-trioxolane proton ring (Soriano et al., 2003; Diaz et al., 2005).

The comparison of the ^{13}C NMR spectrum between RBO and ozonated RBO at pH 4 with ozone dosage 440 gr of O_3/L shows that some of the chemical shifts of the key components involved in the ozonation reaction can be detected in each signal. RBO is a combination of triglycerides and the unsaturated fatty acids oleic, linoleic, and linolenic acids. The RBO sample without ozonation forms 8 signals in the ^{13}C NMR spectrum data shown in Figure 8a. The presence of ozonide chemicals is indicated by signals B (130.15 ppm) and C (128 ppm) as shown in Figure 8b. Signal B with a value of 130.15 ppm became an indicator for oleic acid chemical shift, whereas signal C with a value of 128 ppm became an indicator for linoleic acid chemical shift. The presence of Carbonyl (ester)

(RCOOR') is indicated by Signal A, which is in the range = 172 – 174 ppm. The presence of glycerol molecules is indicated by the D, E, and F signals, which are in the range = 60 – 70 ppm. Meanwhile, the G & H signal in the 10–35 ppm range shows the presence of aliphatic carbons as well as methyl molecules. Carbon intensity decreases significantly in the chemical shift areas of signals B and C. Both signals signaling oleic and linoleic unsaturated fatty acids appear to diminish following ozonation. This implies that ozone effectively attached some of the double bonds seen in RBO derived from oleic and linoleic acids. The chemical changes for RBO and ozonated RBO are similar and just differ in carbon intensity. The data on the ^{13}C NMR results are directly proportional to the ^1H NMR data results, where the B & C signals (130.15 & 128 ppm) are the same as the point J in ^1H NMR data in the 5-ppm range, indicating oleic and linoleic acids. The D,E,F signal (60-70 ppm) was the same as the G & H ^1H NMR signal in the 4 ppm range, suggesting glycerol and triglycerides.

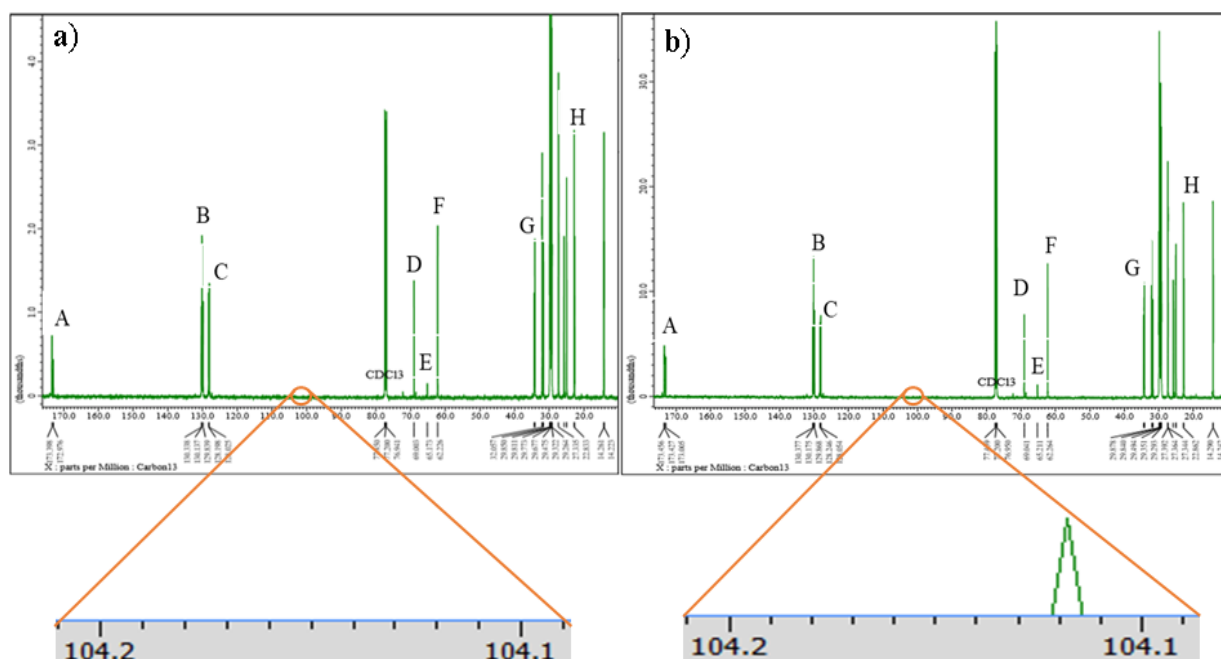


Figure 8. The ^{13}C NMR spectra for RBO a) before and b) after ozonation process.

The G & H signal (10 – 35 ppm) is the same as the A,B,C,D,E ^1H NMR signal, which shows methyl compounds and compounds with CH_2 bonds linked to carbonyl or not. The spectra NMR of ozonated and non-ozonated are similar, however the ozonated sample have another peak after expand of the spectrum. **Figure 6** and **7** is a new chemical shift signal detected in the RBO after ozonation (ozonated RBO) with a range of δ_{H} 5.13 to 5.18 ppm and δ_{C} 104.1 – 104.2 ppm.

GCMS Analysis

From **Table 2**, it can be seen that the composition of linolenic acid (C18:3) and linoleic acid (C18:2) was significantly reduced after RBO was ozonated at an ozone dose of 440 mg O_3/L oil at both pH 3 and 4, while oleic acid (C18:1) increased after the RBO was ozonated. So that linoleic acid (C18:3) and linoleic acid (C18:2) oxidized faster. The same trend also occurs in the ozonation of olive oil and sunflower oil, where the ozonation occurs gradually according to the adequacy of the ozone gas reactant and after ozonation the content of linoleic acid (C18:2) is greater than that of oleic acid (C18:1) (Diaz et al., 2005). The cleavage of the C double bond in linolenic acid (C18:3) and linoleic acid (C18:2) causes an increase in the amount of C18:1 and saturated fatty acids such as; Myristic acid (C14:0), Palmitic (C16:0) and stearic acid (C18:0). Lauric acid (C12:0) in RBO tends to be depleted after the ozonation process at pH 3 and 4. When viewed from the molecular structure of fatty acid has a non-polar hydrocarbon group on the tail and a polar carboxylate group ($-\text{COOH}$) on the head so that it can interact with water from ascorbic acid solution in RBO and carbonyl atoms can be

attacked by nucleophile ozone so that lauric acid will change its structure.

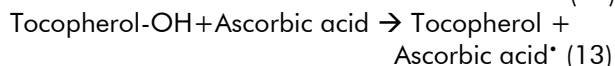
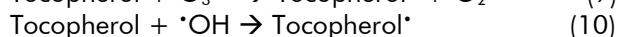
Antioxidant activity and future prospect

Antioxidants are substances that inhibit oxidative processes by acting as antioxidants. Antioxidants include vitamin E (tocopherol & tocotrienol) which contained in RBO and additional ascorbic acid (Colunga et al., 2020; Schwartz et al., 2008). The purpose of adding ascorbic acid is to lower the pH so that ozone is more stable and reacts more readily with the C double bonds in the fatty acids in the RBO. The addition of ascorbate solution is intended to reduce the mixture's pH (emulsion of water, oil and ascorbate). In the meanwhile, pH influences the decomposition of ozone because, as pH rises, the number of OH ions increase, accelerating the ozone decomposition process, and decreasing the quantity of ozone in interaction with saturated fatty acids. Additionally, the antioxidant properties of ascorbic acid and tocopherol can add value to ozonated RBO in addition to the ozonide and peroxide content. Vitamins C and E, which are antioxidants, are beneficial for health and skin maintenance. The variables with the highest tocopherol levels are likewise high in oleic acid. This suggests that the presence of tocopherol as an ozone oxidation barrier prevents ozone from oxidizing oleic unsaturated fatty acids. The tocopherol data from the HPLC test did not show a significant change, although it tended to decrease after ozonation. This is due to the synergy of Vitamin C (ascorbate) with vitamin E in the oil (Packer et al., 1979), in instances when vitamin E is likely to be oxidized by ozone and form vitamin E radicals, which are then regenerated by vitamin C to become vitamin E, the following process might be

Table 3. Saturated and Unsaturated Fatty Acids of RBO before and after ozonation process

Composition	Before	After (pH 3)	After (pH 4)
Saturated			
C10:0 (%)	0.08	0.00	0.08
C12:0 (%)	0.15	0.00	0.00
C14:0 (%)	0.41	0.44	0.42
C16:0 (%)	17.2	18.1	18.0
C18:0 (%)	2.07	2.12	2.00
Total (%)	19.91	20.66	20.5
Unsaturated			
C18:1 (%)	37.1	39.7	39.6
C18:2 (%)	40.1	36.4	36.0
C18:3 (%)	0.95	0.9	0.88
Total (%)	78.15	77	76.48
Tocopherol (mg/100 g)	8.06	8.16	8.05

followed:



Vitamin E (tocopherol) functions primarily as a chain-breaking antioxidant and prevents the formation of lipid peroxidation by oxidizing agents (Poston, Chappell, Seed, & Shennan, 2011; Burton et al., 1985). Vitamins E and C are non-enzymatic antioxidants that have small molecules, Vitamin E is fat-soluble, while vitamin C is water-soluble. Vitamins C and E can neutralize reactive oxygen species (ROS) in a process called radical scavenging and carry them away (Nimse & Pal, 2015).

CONCLUSIONS

The RBO's chemical composition changed as a result of ozonation. Despite a reduction in unsaturated acids, several new compounds were identified in the ozonated RBO. The chemical and physical properties of RBO with and without the addition of ascorbic acid as an ozone stabilizer and pH adjuster were compared. As the pH increased, the viscosity, density, AN, and IN increased. The PN, on the other hand, decreased. Furthermore, as the ozone dose was increased, all parameters were increased. The optimum pH for the RBO ozonation process in this research was pH 4, and the best ozone dosage was 440 mg O₃/L because it has a greater percentage of IN reduction. NMR spectra changes confirm chain scission at C=C bonds in fatty acid chains and the production of novel carbonyl compounds such as aldehyde, 1,2,4-trioxolane, ozonide, and hydroxyl derivatives. Since the excessive use of antibiotics for the treatment of infectious illnesses, ozonated RBO has emerged as a viable and environmentally acceptable alternative.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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