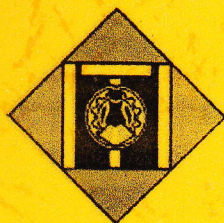


LAPORAN PENELITIAN

PENELITIAN DASAR



JUDUL PENELITIAN:

**SINTESIS TRIOXOLANE DAN DIPEROKSIDA DARI OZONASI ASAM LEMAK
TIDAK JENUH DALAM RICE BRAND OIL(RBO) SEBAGAI BAHAN AKTIF
OBAT HIV/AIDS**

Tim Peneliti :

Ketua :

Dr. Ir. Enjarlis, MT (0308086404)

Anggota :

1. Dr. Sri Handayani (0315106501)

2. Dr. Sofa Fajriah (197418112007012006)

**Dibiayai oleh Direktorat Jenderal Pendidikan Tinggi, Kementerian Pendidikan dan
Kebudayaan, No.4/AKM/PNT/2019 dan No.059/KP/LPKT-ITI/IV/2019.**

INSTITUT TEKNOLOGI INDONESIA (ITI)

2 Januari 2020

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INSTITUT TEKNOLOGI INDONESIA (ITI)

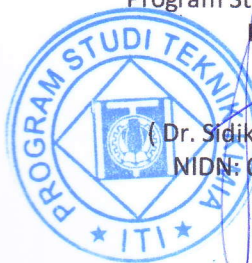
2 Januari 2020

HALAMAN PENGESAHAN

- I. **Judul Pengabdian** : **SINTESIS TRIOXOLANE DAN DIPEROKSIDA DARI OZONASI ASAM LEMAK TIDAK JENUH DALAM RICE BRAND OIL(RBO) SEBAGAI BAHAN AKTIF OBAT HIV/AIDS**
- II. **Bidang Fokus** : **Obat dan Kesehatan**
- III. **Kode/ Nama Rumpun Ilmu** : **433/Teknik Kimia**
- A. PENELITI** :
- a. **Nama Lengkap** : **Dr. Enjarlis, MT**
- b. **NIDN** : **0308086404**
- c. **Jabatan Fungsional** : **Lektor Kepala (700)**
- d. **Program Studi** : **Teknik Kimia**
- e. **Nomor Hp** : **081381234418**
- f. **Alamat Surel (e-mail)** : **en.jarlis@iti.ac.id atau en_jarlis@yahoo.com**
- B. ANGGOTA I** :
- a. **Nama Lengkap** : **Dr. Sri Handayani, MT**
- b. **NIDN** : **0315106501**
- c. **Jabatan Fungsional** : **Lektor Kepala (400)**
- d. **Program Studi** : **Teknik Kimia**
- e. **Nomor Hp** : **08128327412**
- f. **Perguruan Tinggi** : **Institut Teknologi Indonesia**
- C. ANGGOTA II** :
- a. **Nama Lengkap** : **Dr. Sofa Fajriah, MSi**
- b. **Institusi** : **LIPI KIMIA**
- c. **Nomor Hp** : **081317671981**
- D MITRA** :
- i. **Sumber Dana** : **LISTEK DIKTI**
- n. **Biaya Penelitian** : **260.151,500juta**

Tangerang Selatan, 2 Januari 2020

Mengetahui
Program Studi Teknik Kimia
Ketua



(Dr. Sidik Marsudi, MT)
NIDN: 0317036603

Ketua Tim

(Dr. Enjarlis, MT)
NIDN : 0308086404

Mengetahui,
Lembaga Penelitian dan
Komersialisasi Teknologi Indonesia (LPKT) ITI



Direktur
(Dr. Ir. Joelianingsih., MSi)
NIDN: 0310076406

PROTEKSI ISI LAPORAN KEMAJUAN PENELITIAN

Dilarang menyalin, menyimpan, memperbanyak sebagian atau seluruh isi laporan ini dalam bentuk apapun kecuali oleh peneliti dan pengelola administrasi penelitian

LAPORAN KEMAJUAN PENELITIAN MULTI TAHUN

ID Proposal: 0edfcbc7-838b-4dc7-bbd7-6e72e5f9db07
Laporan Kemajuan Penelitian: tahun ke-1 dari 2 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

SINTESIS TRIOXOLANE DAN DIPEROKSIDA DARI OZONASI ASAM LEMAK TIDAK JENUH DALAM RICE BRAND OIL(RBO) SEBAGAI BAHAN AKTIF OBAT HIV/AIDS

B. BIDANG, TEMA, TOPIK, DAN RUMPUN BIDANG ILMU

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kesehatan	Teknologi kemandirian bahan baku obat	Bahan baku obat kimia	Farmasetika dan Teknologi Farmasi

C. KATEGORI, SKEMA, SBK, TARGET TKT DAN LAMA PENELITIAN

Kategori (Kompetitif Nasional/ Desentralisasi/ Penugasan)	Skema Penelitian	Strata (Dasar/ Terapan/ Pengembangan)	SBK (Dasar, Terapan, Pengembangan)	Target Akhir TKT	Lama Penelitian (Tahun)
Penelitian Kompetitif Nasional	Penelitian Dasar	SBK Riset Dasar	SBK Riset Dasar	3	2

2. IDENTITAS PENGUSUL

Nama, Peran	Perguruan Tinggi/ Institusi	Program Studi/ Bagian	Bidang Tugas	ID Sinta	H-Index
ENJARLIS Ketua Pengusul	Institut Teknologi Indonesia	Teknik Kimia		5998045	0
Dr SRI HANDAYANI S.T, M.T Anggota Pengusul 1	Institut Teknologi Indonesia	Teknik Kimia	Bertanggung jawab dalam mempersiapkan dan bahan baku Minyak Nabati yang berkualitas dengan kemurnian yang tinggi pada karakteristik tertentu. Persiapan bahan baku meliputi Pemurnian dan karakterisasinya	6015740	0

			RBO, dan membantu uji-uji fisika dan kimia produk dan bahan baku RBO serta terhadap keluaran berupa publikasi di jurnal internasional.		
Dr. Sofa Fajriah, M.Si Anggota Pengusul 2	LIPI KIMIA	-	bertanggung jawab atas pelaksanaan analisa yang terkait, identifikasi produk yang mengandung senyawa aktif secara kimia (analisa bilangan asam, peroksida, lod, komposisi dengan GC-MS, gugus fungsi menggunakan FTIR dan NMR serta evaluasi hasil uji in vitro & in Vivo produk serta uji toksisitas	0	0

3. MITRA KERJASAMA PENELITIAN (JIKA ADA)

Pelaksanaan penelitian dapat melibatkan mitra kerjasama, yaitu mitra kerjasama dalam melaksanakan penelitian, mitra sebagai calon pengguna hasil penelitian, atau mitra investor

Mitra	Nama Mitra
-------	------------

4. LUARAN DAN TARGET CAPAIAN

Luaran Wajib

Tahun Luaran	Jenis Luaran	Status target capaian (<i>accepted, published, terdaftar atau granted, atau status lainnya</i>)	Keterangan (<i>url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya</i>)
1	Publikasi Ilmiah Jurnal Internasional	accepted/published	Jurnal Internasional Chemical Science

Luaran Tambahan

Tahun Luaran	Jenis Luaran	Status target capaian (<i>accepted, published, terdaftar atau granted, atau status lainnya</i>)	Keterangan (<i>url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya</i>)
1	Publikasi Ilmiah Jurnal Internasional	accepted/published	International Journal of Engineering and Technology(UAE) - Scopus
1	Prosiding dalam pertemuan ilmiah Internasional	sudah terbit/sudah dilaksanakan	12th Annual Congress on Chemistry

5. ANGGARAN

Rencana anggaran biaya penelitian mengacu pada PMK yang berlaku dengan besaran minimum dan maksimum sebagaimana diatur pada buku Panduan Penelitian dan Pengabdian kepada Masyarakat Edisi 12.

Total RAB 2 Tahun Rp. 536,305,502

Tahun 1 Total Rp. 260,151,500

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Analisis Data	HR Pengolah Data	P (penelitian)	1	1,540,000	1,540,000
Analisis Data	Biaya analisis sampel	Unit	1	81,377,500	81,377,500
Analisis Data	Tiket	OK (kali)	2	1,300,000	2,600,000
Analisis Data	Penginapan	OH	2	845,000	1,690,000
Analisis Data	Uang Harian	OH	3	462,000	1,386,000
Analisis Data	Transport Lokal	OK (kali)	10	110,000	1,100,000
Analisis Data	Biaya konsumsi rapat	OH	13	63,000	819,000
Analisis Data	Honorarium narasumber	OJ	30	250,000	7,500,000
Bahan	ATK	Paket	1	2,000,000	2,000,000
Bahan	Bahan Penelitian (Habis Pakai)	Unit	1	34,952,000	34,952,000
Bahan	Barang Persediaan	Unit	1	52,000,000	52,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya seminar internasional	Paket	1	4,267,000	4,267,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Uang harian rapat di luar kantor	OH	2	3,500,000	7,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Publikasi artikel di Jurnal Internasional	Paket	2	7,000,000	14,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Uang harian rapat di dalam kantor	OH	10	110,000	1,100,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya konsumsi rapat	OH	12	63,000	756,000
Pengumpulan Data	FGD persiapan penelitian	Paket	1	8,750,000	8,750,000
Pengumpulan Data	Transport	OK (kali)	2	430,000	860,000
Pengumpulan Data	Uang harian rapat di luar kantor	OH	2	462,000	924,000
Pengumpulan Data	HR Sekretariat/Administrasi Peneliti	OB	6	300,000	1,800,000
Pengumpulan Data	Uang harian rapat di dalam kantor	OH	10	110,000	1,100,000
Pengumpulan Data	Biaya konsumsi	OH	10	63,000	630,000
Pengumpulan Data	HR Pembantu Peneliti	OJ	16	2,000,000	32,000,000

Tahun 2 Total Rp. 276,154,002

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Analisis Data	HR Pengolah Data	P (penelitian)	1	1,540,000	1,540,000
Analisis Data	Biaya analisis sampel	Unit	1	104,877,500	104,877,500
Analisis Data	Tiket	OK (kali)	2	1,000,000	2,000,000
Analisis Data	Penginapan	OH	2	845,000	1,690,000
Analisis Data	Uang Harian	OH	6	462,000	2,772,000
Analisis Data	Transport Lokal	OK (kali)	10	110,000	1,100,000
Analisis Data	Biaya konsumsi rapat	OH	16	63,000	1,008,000
Analisis Data	Honorarium narasumber	OJ	30	250,000	7,500,000
Bahan	ATK	Paket	1	2,000,000	2,000,000
Bahan	Bahan Penelitian (Habis Pakai)	Unit	1	52,614,000	52,614,000
Bahan	Barang Persediaan	Unit	1	25,000,000	25,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya seminar internasional	Paket	1	6,032,502	6,032,502
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Publikasi artikel di Jurnal Internasional	Paket	1	7,000,000	7,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Luaran KI (paten, hak cipta dll)	Paket	1	4,000,000	4,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Uang harian rapat di luar kantor	OH	2	3,500,000	7,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Uang harian rapat di dalam kantor	OH	10	110,000	1,100,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya konsumsi rapat	OH	10	63,000	630,000
Pengumpulan Data	FGD persiapan penelitian	Paket	1	8,750,000	8,750,000
Pengumpulan Data	Transport	OK (kali)	2	430,000	860,000
Pengumpulan Data	HR Sekretariat/Administrasi Peneliti	OB	6	300,000	1,800,000
Pengumpulan Data	Uang harian rapat di luar kantor	OH	6	462,000	2,772,000
Pengumpulan Data	Uang harian rapat di dalam kantor	OH	10	110,000	1,100,000
Pengumpulan Data	HR Pembantu Peneliti	OJ	16	2,000,000	32,000,000
Pengumpulan Data	Biaya konsumsi	OH	16	63,000	1,008,000

6. KEMAJUAN PENELITIAN

A. RINGKASAN: Tuliskan secara ringkas latar belakang penelitian, tujuan dan tahapan metode penelitian, luaran yang ditargetkan, serta uraian TKT penelitian.

Latar Belakang dan Tujuan Penelitian

Indonesia negara perkembangan epidemi HIV/AIDS tercepat dan jumlah penderita ke dua terbesar di Asia, rentang usia penderita 20-29 tahun sebanyak 48,1% dan 30-39 tahun sebanyak 30,9%. Penangan penderita HIV/AIDS membutuhkan obat dan biaya cukup besar, karena obat yang ada dipasarannya masih impor seperti ARV. Pengembang obat HIV/AIDS berbasis bahan baku lokal dilakukan untuk mendapatkan zat aktif sebagai anti virus HIV/AIDS. se Zat aktif lain yang ampuh mengurangi virus HIV/AIDS dan mikroorganisme penyebab penyakit lainnya (bakteri dan fungi) adalah Trioxolane dan diperoksida, dimana zat aktif ini dapat disintesis dari bahan baku lokal minyak dedak padi (rice Brand Oil/RBO). RBO dapat diperoleh melalui proses ekstraksi dedak padi menggunakan pelarut organik dan RBO ini mengandung $\pm 75\%$ asam lemak tidak jenuh (Asam oleat/C18:1 38,4%, Asam linoleat/C18:2 sebesar 34,4% dan α Asam Linoleat/C18:3 sebesar 2,2%). Tingginya kandungan asam lemak tidak dalam RBO, maka RBO sangat potensial sebagai bahan baku untuk sintesis zat aktif Trioxolane, peroksida dan turunannya melalui proses ozonasi.

Tujuan penelitian ini yaitu menghasilkan paket teknologi proses sintesis Trioxolane, diperoksida dan turunannya dari bahan baku RBO sebagai zat aktif obat HIV/AIDS atau obat untuk penyakit lainnya yang disebabkan oleh mikroorganisme melalui proses ozonasi.

Tahapan metoda penelitian pada tahun I meliputi:

- (1) Optimasi stabilisasi dedak padi sebelum ekstraksi, sehingga dihasilkan RBO yang berkualitas baik sebagai bahan baku untuk sintesis Trioxolane, peroksida dan turunannya melalui proses ozonasi,
- (2) Optimasi waktu (2 jam, 3 jam dan 4 jam) proses ekstraksi RBO dari dedak padi.
- (3) Optimasi pH (2, 3 dan 4) proses Ozonasi RBO dan kondisi (waktu, jenis katalis, dosis ozon) proses ozonasi.
- (4) Karakterisasi RBO zone secara fisika dan kimi serta terkait kandungan Trioxolane, diperoksida dan turunannya pada ozonasi RBO skala Lab.
- (5) Uji Coba RBO zone sebagai anti Mikroorganisme baru pada bakteri (E Coli, S Aureus dan pseudomonas Aeruginosa) dan anti Jamur (Candida Albicans).

Penelitian Sintesis Trioxolane dan diperoksida dari Ozonasi RBO dimulai dari TKT 2, untuk mencapai TKT 3 sudah dilakukan 5 tahapan penelitian seperti tahapan metodologi penelitian di atas. Dengan demikian sudah di peroleh bahan baku RBO yang berkualitas baik, kondisi proses ozonasi RBO optimal sehingga di hasilkan RBO zone dengan spesifikasi sebagai anti mikroorganisme. Parameter hasil uji RBO Zone yang optimal di hasilkan, ternyata belum memenuhi target yang diharapkan seperti RBO Zone yang dihasilkan : belum mencapai bilangan Iod mendekati Nol, angka Peroksida belum mencapai angka 300 sd 500 Eq O₂/kg Oil dan aksi RBO Zone sebagai anti bakteri pada uji in vitro belum memberikan efek yang signifikan.

B. KATA KUNCI: Tuliskan maksimal 5 kata kunci.

Rice Brand Oil, Stabilisasi Dedak Padi, Ozonasi, Trioxolane dan Peroksida

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan ringkas mungkin. Dilarang menghapus/modifikasi template ataupun menghapus penjelasan di setiap poin.

C. HASIL PELAKSANAAN PENELITIAN: Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian dapat berupa data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan ringkas mungkin. Dilarang menghapus/modifikasi template ataupun menghapus penjelasan di setiap poin.

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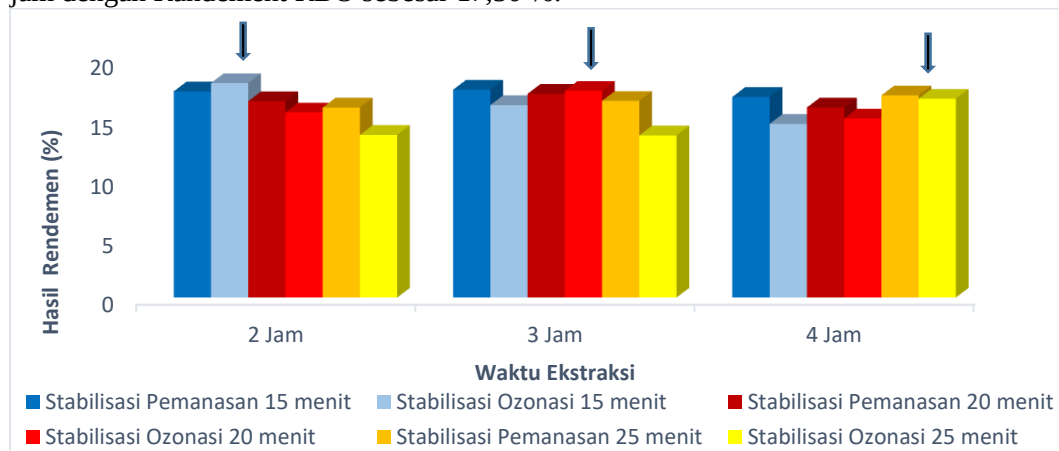
Penelitian yang sudah dilakukan (1) Optimasi stabilisasi dedak Padi dan ekstraksi RBO dari dedak Padi, (2) Optimasi pH dan dosis Ozon pada ozonasi RBO komersiel, (3) Karakterisasi Produk/RBO terozon dan uji terhadap bakteri.

1. Stabilisasi Dedak Padi

Stabilisasi dedak padi dilakukan menggunakan metode pemanasan dan ozonisasi, tujuan stabilisasi adalah untuk mentidak aktifkan enzim Lipase dalam dedak padi yang dapat menyebabkan RBO rusak dan yiel RBO berkurang.

Rendemen RBO:

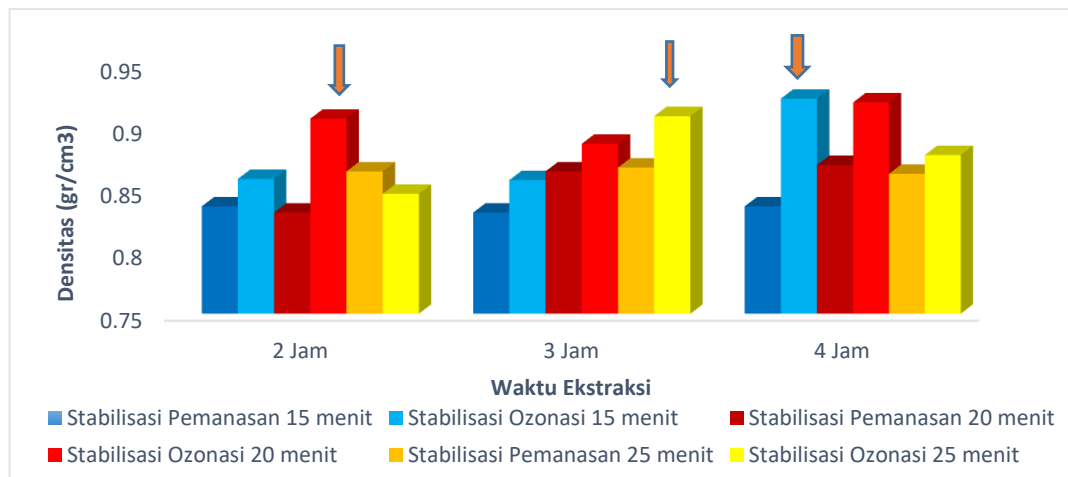
Gambar 1 adalah pengaruh waktu (15, 20 dan 25 menit) stabilisasi, metode stabilisasi (pemanasan dan ozonisasi) dan waktu ekstraksi RBO terhadap nilai Randemen yang dihasilkan. Dari grafik diketahui bahwa stabilisasi dengan metode ozonasi selama 15 menit dan ekstraksi selama 2 jam ($\text{..gr O}_3/\text{ml.hr}$) menghasilkan randemen sebesar 18,06 %, lebih baik dibandingkan stabilisasi menggunakan metode pemanasan. Stabilisasi dedak padi dengan metode pemanasan terbaik terjadi pada waktu stabilisasi di 15 menit dan ekstraksi selama 3 jam dengan Randement RBO sebesar 17,50 %.



Gambar 1. Pengaruh Metode Stabilisasi dan Waktu Ekstraksi Terhadap Hasil Persen Rendemen

Densitas RBO

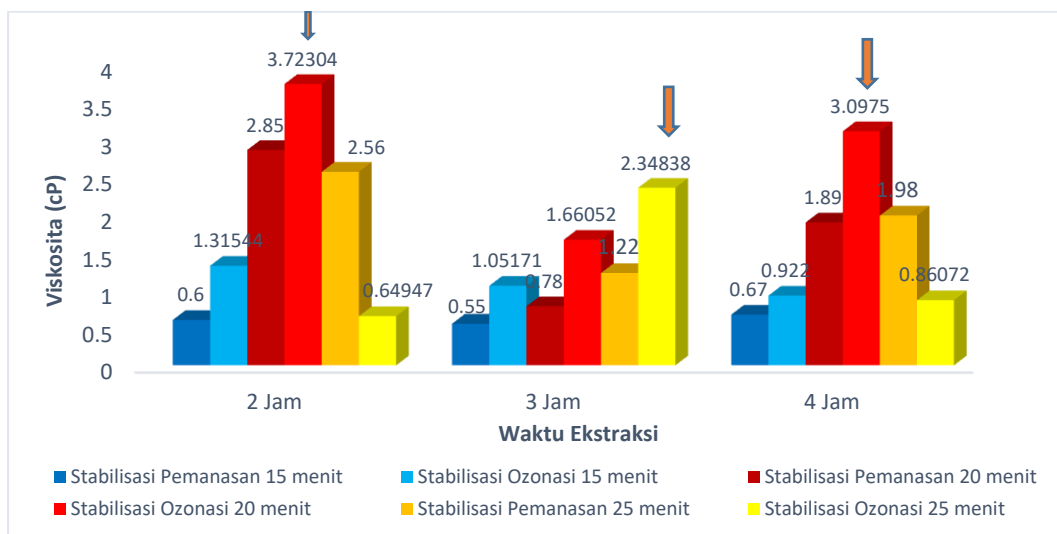
Gambar 2. Adalah Pengaruh waktu (15, 20 dan 25 menit) stabilisasi, metode stabilisasi (pemanasan dan ozonisasi) dan waktu ekstraksi RBO terhadap nilai densitas. Dari gambar ini teramati bahwa stabilisasi dengan metode ozonasi selama 20 menit dan ekstraksi selama 2 jam memberikan nilai densitas RBO sebesar 0,906 gr/ml **lebih besar** dibandingkan dengan metode pemanasan yang memberikan densitas 0,867 gr/ml. Hal yang sama juga terjadi pada stabisasi dengan metode ozonasi selama 20 menit dan ekstraksi selama 4 jam memberikan nilai densitas lebih besar dibandingkan dengan metode stbilisasi dengan metode pemanasan yaitu dengan nilai densitas sebesar 0,922 gr/ml(metodo ozonasi) dan 0,869 gr/ml (metode pemanasan).



Gambar 2. Pengaruh Metode Stabilisasi dan Waktu Ekstraksi Terhadap Hasil Densitas

Viscositas RBO

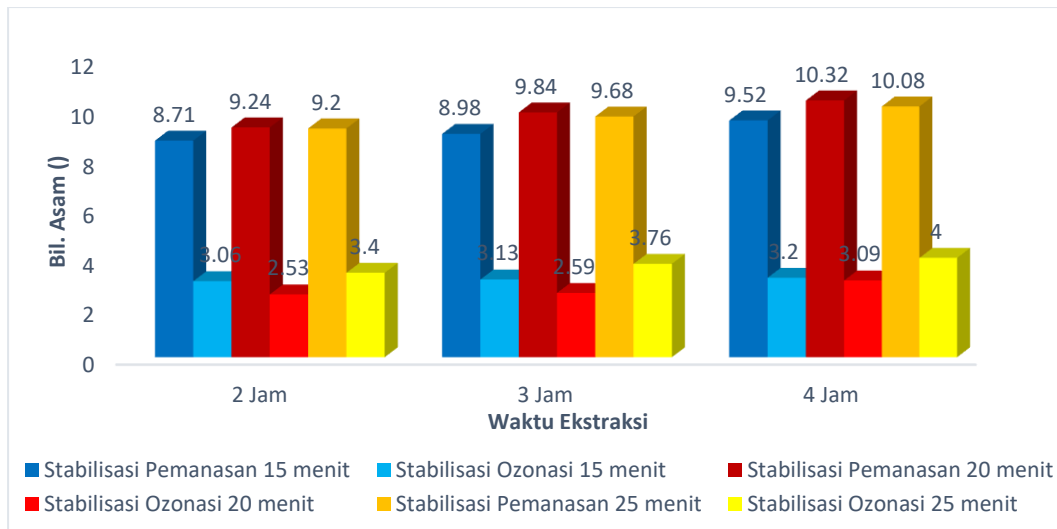
Gambar 3. Adalah Pengaruh waktu (15, 20 dan 25 menit) stabilisasi, metode stabilisasi (pemanasan dan ozonisasi) dan waktu ekstraksi RBO terhadap nilai Viskositas. Dari kurva di amati bahwa nilai viskositas tertinggi terjadi pada metode stabilisasi dedak dengan menggunakan metode ozonasi.



Gambar 3 Pengaruh Metode Stabilisasi dan Waktu Ekstraksi Terhadap Hasil Viskositas

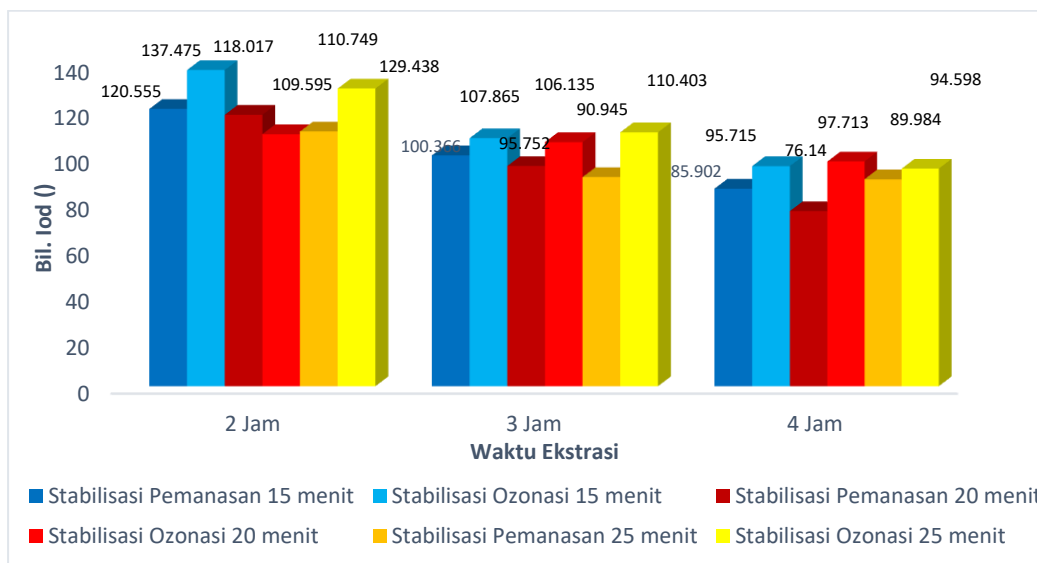
Bilangan Asam

Gambar 4. Adalah Pengaruh waktu (15, 20 dan 25 menit) stabilisasi, metode stabilisasi (pemanasan dan ozonisasi) dan waktu ekstraksi RBO terhadap nilai bilangan asam. Dari Gambar 4 terlihat bahwa nilai bilangan asam RBO terendah terjadi pada metode stabilisasi dengan menggunakan ozonasi baik ekstraksi dilakukan 2, 3 dan 4 jam dibandingkan dengan metode pemanasan. Hal ini menunjukkan stabilisasi dapat memperkecil asam bebas dan berarti sangat efektif dalam merusak enzim Lipase.



Gambar 4 Pengaruh Metode Stabilisasi dan Waktu Ekstraksi Terhadap Hasil Analisa Bilangan Asam
Bilangan Iodium>

Gambar 5. Adalah Pengaruh waktu (15, 20 dan 25 menit) stabilisasi, metode stabilisasi (pemanasan dan ozonisasi) dan waktu ekstraksi RBO terhadap nilai bilangan Iod. Dari gambar 5 teramati bahwa nilai bilangan iod tertinggi terjadi pada stabilisasi menggunakan ozonasi selama 15 menit dan ekstraksi selama 2 jam dengan nilai bilangan Iod sebesar 137,475 gr Iod/kg Oil. Tingginya bilangan Iod RBO yang dihasilkan dari ekstraksi dedak padi menggunakan stsbilisasi, maka menunjukan metodo ozonasi lebih tepat dalam stabilisasi dedak padi sebelum diekstraksi.

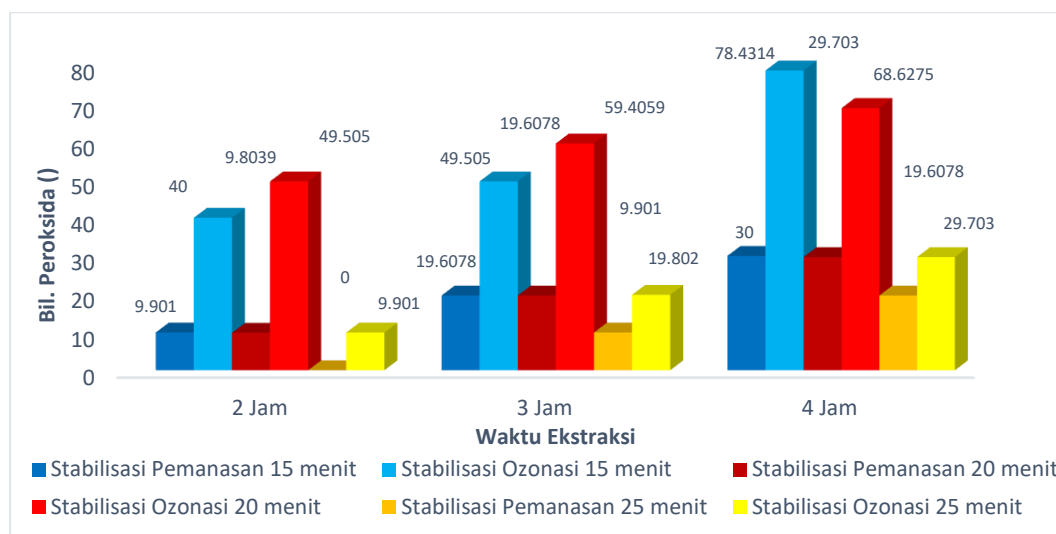


Gambar 5 Pengaruh Metode Stabilisasi dan Waktu Ekstraksi Terhadap Hasil Bilangan Iod

Bilangan Peroksida

Gambar 6. Adalah Pengaruh waktu (15, 20 dan 25 menit) stabilisasi, metode stabilisasi (pemanasan dan ozonisasi) dan waktu ekstraksi RBO terhadap nilai bilangan Peroksida. Dari gambar 6 teramati bahwa nilai bilangan peroksida tertinggi terjadi pada stabilisasi menggunakan ozonasi selama 20 menit dan ekstraksi selama 4 jam dengan nilai bilangan peroksida sebesar 78,43 gr Eq O₂/kg Oil dan pada stabilisasi pemanasan nilai bilangan Peroksida RBO sebesar 68,63 g Eq₂/kg Oil. Sedangkan Bilangan peroksida terendah

terjadi pada stabilisasi dengan metode ozonasi maupun pemanasan terjadi pada stabilisasi selama 15 menit dimana untuk metode stabilisasi dengan pemanasan mencapai 0 dan ozonasi mencapai 9,90.



Gambar 6 Pengaruh Metode Stabilisasi dan Waktu Ekstraksi Terhadap Hasil Bilangan Peroksida

Komposisi Asam Lemak

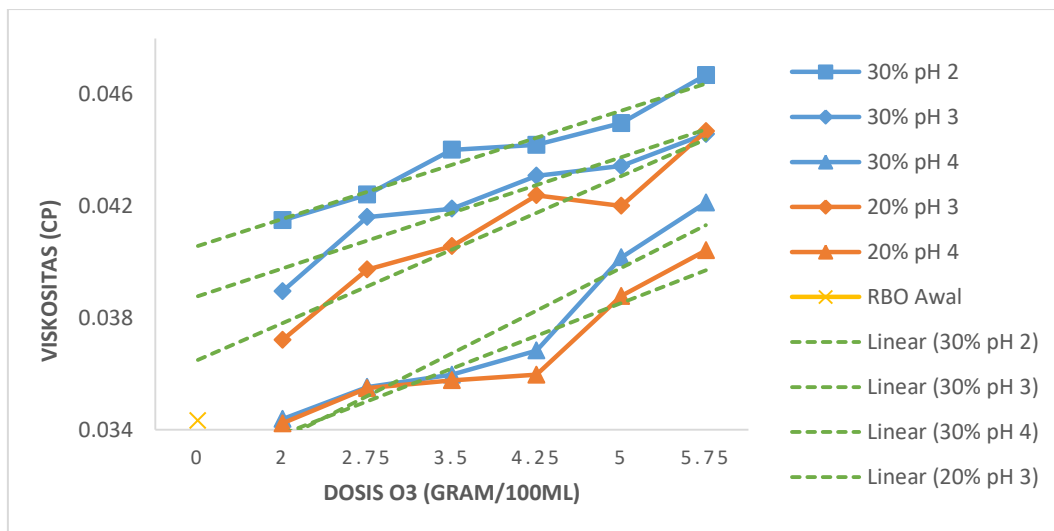
Tabel 1 adalah Data komposisi Asam lemak dalam RBO yang di hasilkan dari Ekstraksi dengan menggunakan stabilisasi menggunakan metode Ozonasi dan Pemanasan. Tabel 1. ini menunjukan bahwa kandungan asam lemah tidak jenuh dalam RBO yang dihasilkan dari metode stabilisasi Ozonasi lebih besar di bandingkan dengan stabilisasi menggunakan metode Pemanasan.

Table 1. Composition of fatty acids from rice bran oil from gas chromatography analysis

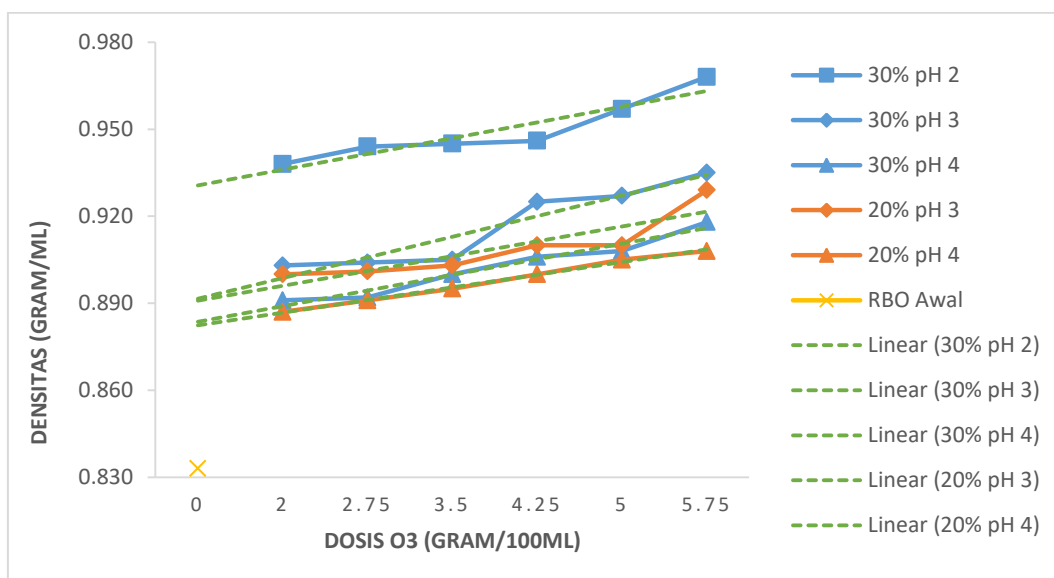
Methyl esters	Method Pemanasan Percentage (%)	Method Ozonasi Precentage (%)
Lauric acid (C12: 0)	0,1	0,1
Myristic acid (C14: 0)	0,9	1,2
Palmitic acid (C16: 0)	18,2	15,4
Stearic acid (C18: 0)	2,6	2,3
Oleic Acid (C18: 1)	36,7	39,5
Linoleic acid (C18: 2)	37,0	38,8
Linolenic acid (C18: 3)	1,2	1,3

2. Optimasi pH dan dosis Ozone pada Ozonasi RBO Komersiel

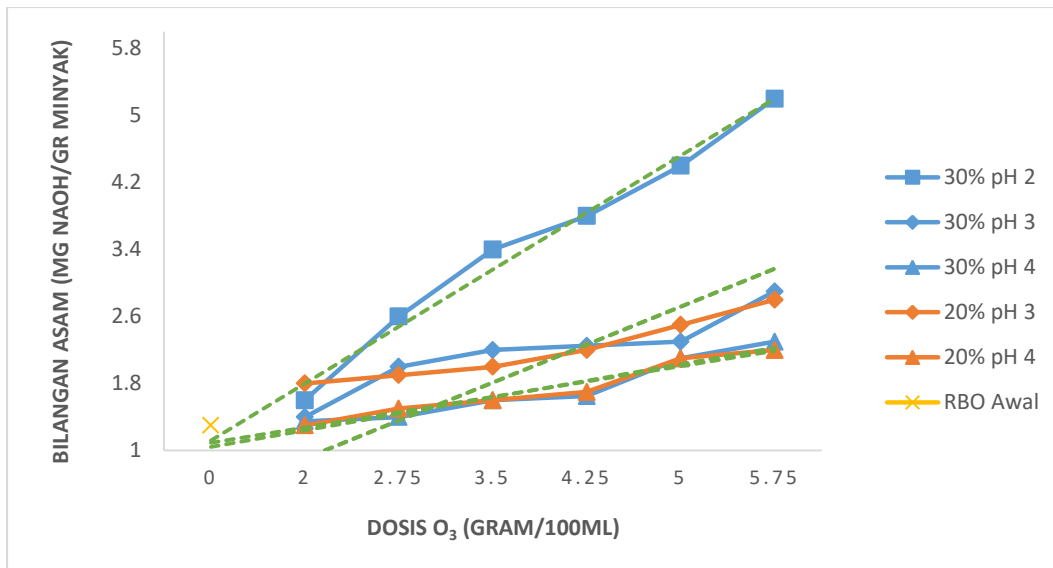
Gambar 6 adalah pengaruh pH (2, 3 dan 4) proses ozonasi RBO pada sintesis zat aktif Trioxolane, diperoksida dan turunannya pada dosis ozon (2 gram, 2,75 gram, 3,5 gram, 4,25 gram, 5 gram dan 5,75 gram dalam 100 ml RBO pada suhu 5°C dengan asam ascorbat (Vitamin C). Dari hasil percobaan pH proses ozonasi terbaik terjadi pada pH 4 dan dosis ozon 5,75 gr, hal ini di dasarkan pada penurunan % bilangan Iod dan kenaikan bilangan Peroksida serta analisa NMR yang menunjukan adanya terbentuk Aldehid dan peroksida pada NMR kelihatan masih sedikit.



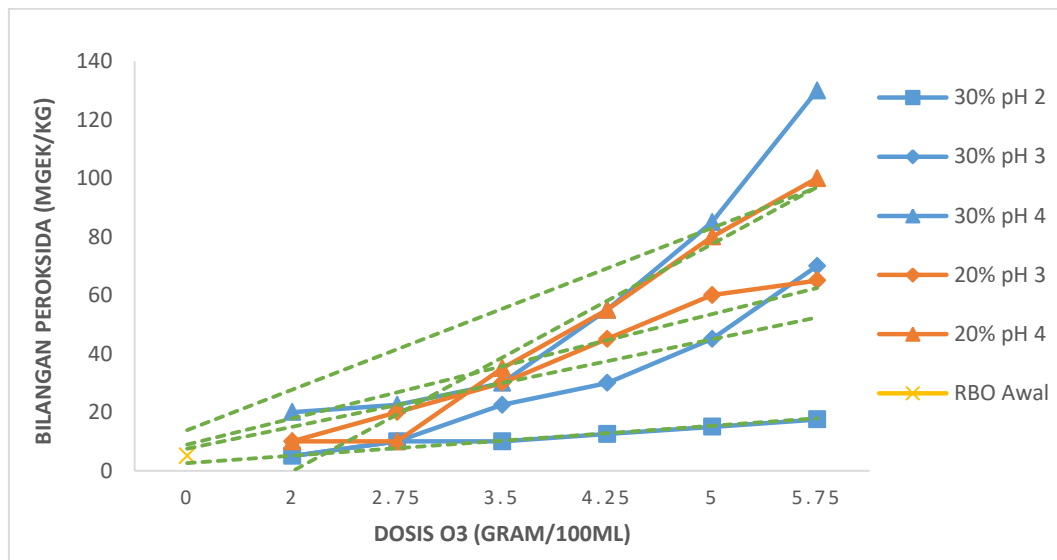
Gambar 7. Pengaruh Dosis Ozon dan pH Proses Ozonasi pada suhu 5°C terhadap Viskositas RBO



Gambar 8 Pengaruh Dosis Ozon dan pH Proses Ozonasi pada suhu 5°C terhadap Densitas RBO



Gambar 9 Pengaruh Dosis Ozon dan pH Proses Ozonasi pada suhu 5°C terhadap Bilangan Asam RBO



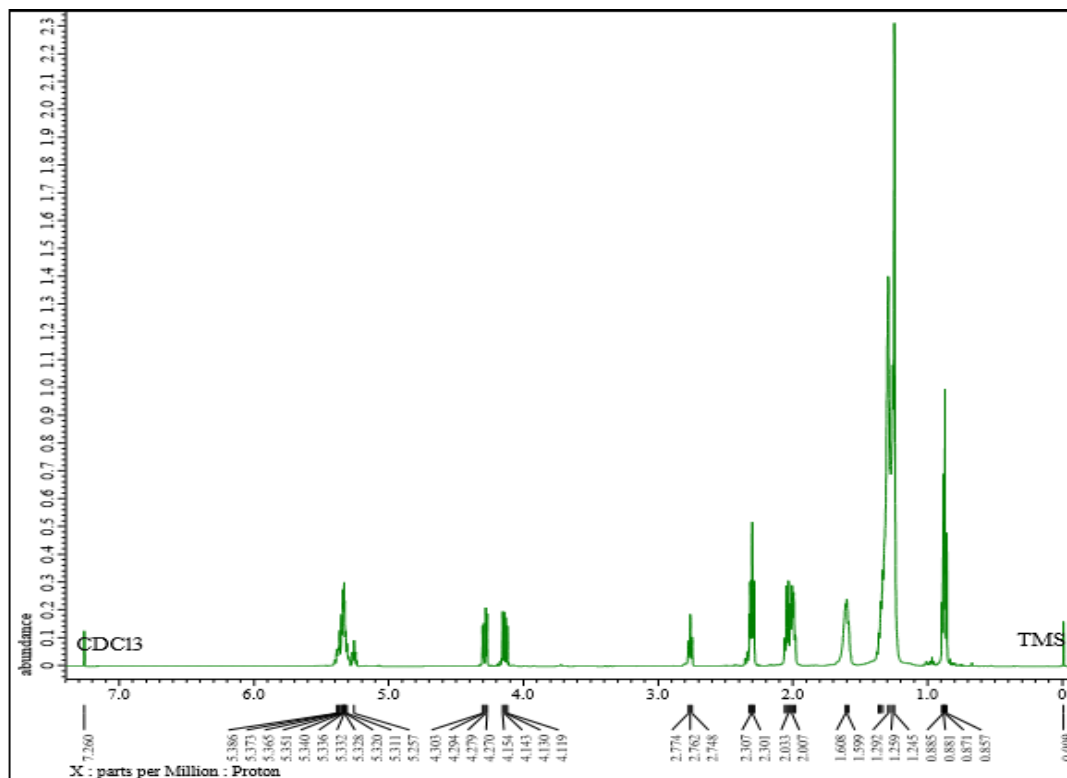
Gambar 10. Pengaruh Dosis Ozon dan pH Proses Ozonasi pada suhu 5°C terhadap Bilangan Peroksida RBO

Tabel 2 Persentase Penurunan Bilangan Iod

Dosis O ₃	Persentase Penurunan Bil.Iod (%)				
	30% pH 2	20% pH 3	30% pH 3	20% pH 4	30% pH 4
2,00	6.03	4.31	37.93	16.38	11.21
2.75	11.21	12.07	84.91	27.59	12.07
3.50	14.91	22.41	49.14	53.45	14.66
4.25	16.38	37.07	56.03	64.66	87.93
5,00	17.24	51.72	56.90	82.76	95.26
5.75	28.88	67.24	80.17	93.10	97.84

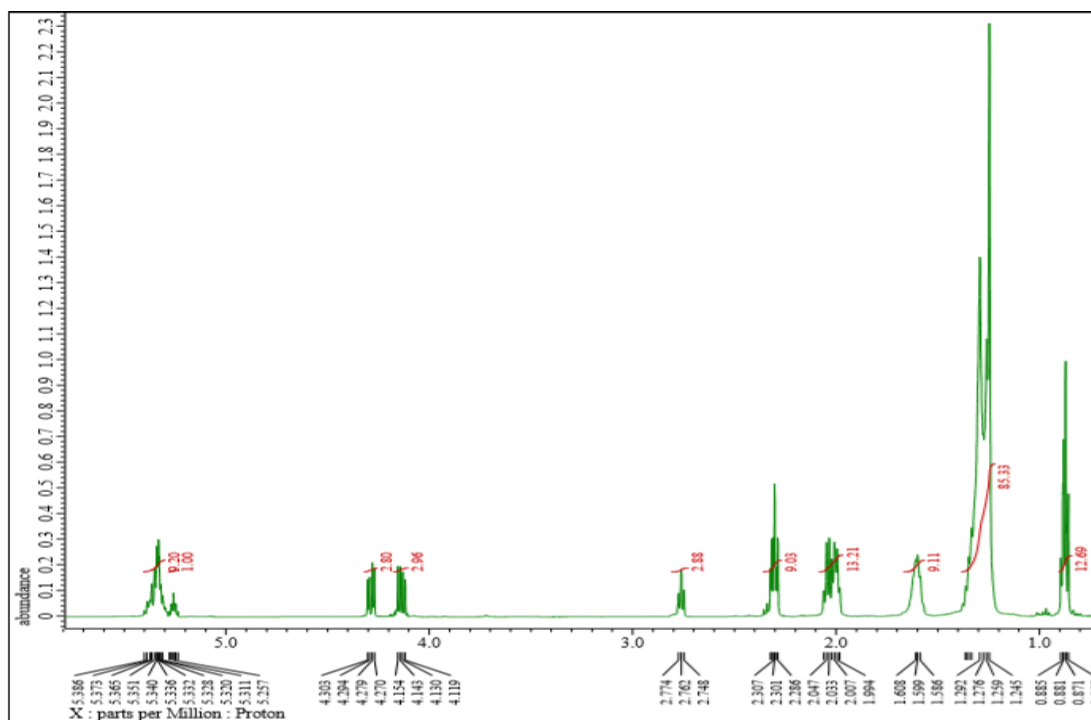
Analisa NMR

Dari gambar 4.11 terlihat bahwa terdapat 10 sinyal pada spektrum ^1H NMR. Sinyal yang nampak tersebut mengindikasikan bahwa ada 10 macam proton (H) yang berbeda dalam senyawa yang menghasilkan spektrum tersebut. Perbedaan dari proton-proton tersebut adalah intensitas yang dimiliki dan geseran kimia yang berbeda-beda.



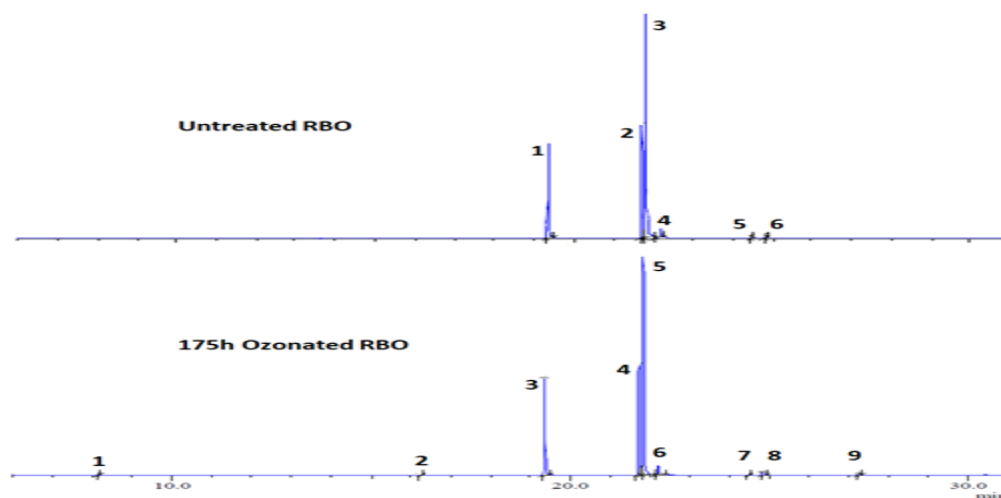
Gambar 11 Spektrum ^1H NMR Rice Bran Oil pH 4 dan $[\text{O}_3]$ 5.75 gram

Dari gambar 4.12 terlihat 10 sinyal proton yang berbeda. Sinyal dengan jumlah proton tertinggi adalah sinyal (b) dengan nilai (85.33). Tingginya integral sinyal proton (b) mengindikasikan jumlah proton yang menghasilkan sinyal (b) lebih banyak dari yang lain, yang diikuti dengan sinyal d(13.21), a(12.69), j(9.2), c(9.11), e(9.03), g(2.96), f(2.88), h(2.8), i(1). Setiap puncak dari setiap sinyal mempunyai chemical shift dan intensitas yang berbeda. Informasi mengenai sinyal, geseran kimia, jumlah proton, jenis proton serta jenis senyawa pada gambar 4.8.1 menunjukan pada $\delta = 5.4$ ppm (j) dengan ditandai sinyal proton olefin menandakan terbentuknya H_2O_2 . Pada $\delta = 2.3$ ppm (CH_2COOH), $\delta = 1.67$ ppm ($\text{CH}_2\text{CH}_2\text{COOH}$), $\delta = 1.32$ ppm (CH_2)_n, (e), (a), (c) menandakan adanya gugus alkanoyl dengan ditandai adanya multiplet dari terbentuknya proton-proton ozonida. Pada $\delta = 4.1 - 4.35$ ppm (CH_2OCOR) triplets menandakan adanya senyawa glycerol. Pada $\delta = 2.76$ ppm dengan ditandai triplet dari proton olefin menandakan adanya asam lemak jenuh linolenat dan linoleat. Pada $\delta = 0.87$ ppm menandakan senyawa grup methyl.



Gambar 12 Spektrum ^1H NMR ozonated oil RBO pH 4 dan $[\text{O}_3]$ 5.75 gram dengan nilai integral

Pengaruh Waktu Reaksi Ozonasi Terhadap Kualitas RBO Terozon



Gambar 13. Hasil Analisa GC MS untuk RBO sebelum diozonasi dan sesudah ozonasi selama 175 jam

Table 3. Chemical component of untreated RBO showed from GCMS analysis

Peak No.	%Area	Retention Time	Molecule name	Formula	Molar mass	Ion Mass (m/z)
1	20.26	19.351	Palmitic acid	$\text{C}_{17}\text{H}_{34}\text{O}_2$	270	74 $[\text{CH}_2\text{CO}_2\text{CH}_3+\text{H}]^+$ 87 $[\text{C}_3\text{H}_7\text{CO}_2]^+$ 43 $[\text{C}_3\text{H}_7, \text{CH}_3\text{C}=\text{O}, \text{C}_2\text{H}_5\text{N}]^+$ 41 $[\text{CH}_2\text{C}=\text{N}, \text{Ar}]^+$ 57 $[\text{C}_4\text{H}_9, \text{C}_2\text{H}_5\text{C}=\text{O}]^+$
2	23.51	21.724	Methyl linoleate	$\text{C}_{19}\text{H}_{34}\text{O}_2$	294	55 $[\text{C}_4\text{H}_7, \text{CH}_2=\text{CHC}=\text{O}]^+$ 67 $[\text{C}_5\text{H}_7]^+$ 81 $[\text{Side Chain Ring-CH}_2, \text{C}_6\text{H}_9]^+$ 41 $[\text{CH}_2\text{C}=\text{N}, \text{Ar}]^+$
3	53.62	21.827	Methyl Oleate	$\text{C}_{19}\text{H}_{36}\text{O}_2$	296	95 $[\text{Side Chain Ring-C}=\text{O}]^+$

						55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 41[CH ₂ C=N, Ar] ⁺ 69[C ₅ H ₉ ,CF ₃ ,CH ₃ CH=CHC=O] ⁺ 97[C ₇ H ₃ ,S-Side Chain Ring-CH ₂] ⁺ 98[O-Side Chain Ring-CH ₂ O+H] ⁺
4	2.03	22.204	Methyl stearate	C ₁₉ H ₃₈ O ₂	530	41[CH ₂ C=N, Ar] ⁺ 69[C ₅ H ₉ ,CF ₃ ,CH ₃ CH=CHC=O] ⁺ 83[C ₆ H ₁₁ ,CH ³⁵ CL ₂] ⁺ 96[(CH ₂) ₅ =N] ⁺ 111[S-Side Chain Ring-C=O] ⁺
5	0.19	24.492	Octacosanoic acid	C ₂₉ H ₅₈ O ₂	530	41[CH ₂ C=N, Ar] ⁺ 69[C ₅ H ₉ ,CF ₃ ,CH ₃ CH=CHC=O] ⁺ 83[C ₆ H ₁₁ ,CH ³⁵ CL ₂] ⁺ 96[(CH ₂) ₅ =N] ⁺ 111[S-Side Chain Ring-C=O] ⁺
6	0.39	24.828	Arachidic acid methyl ester	C ₂₁ H ₄₂ O ₂	490	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺ 41[CH ₂ C=N, Ar] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺

Table 3. Chemical Component of Ozonated RBO Showed from GCMS Analysis

Peak No.	%Area	Retention Time	Molecule Name	Formula	Molar mass	Ion Mass (m/z)*
1	0.23	8.138	1,1-Dimethoxydodecane	C ₁₄ H ₃₀ O ₂	230	71[C ₅ H ₁₁ ,C ₃ H ₇ C=O] 41[CH ₂ C=N, Ar] ⁺ 55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 58[CH ₃ C(=O)CH ₂ +H,C ₂ H ₅ CHNH ₂] 96[(CH ₂) ₅ =N] ⁺
2	0.13	16.249	Methyl myristate	C ₁₅ H ₃₀ O ₂	242	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 41[CH ₂ C=N, Ar] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺
3	20.22	19.356	Palmitic acid	C ₁₇ H ₃₄ O ₂	270	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺ 41[CH ₂ C=N, Ar] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺
4	23.80	21.730	Methyl linoleate	C ₁₉ H ₃₄ O ₂	294	55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 67[C ₅ H ₇] ⁺ 41[CH ₂ C=N, Ar] ⁺ 81[Side Chain Ring-CH ₂ ,C ₆ H ₉] ⁺ 55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 109[Side Chain Ring-C=O]
5	51.97	21.833	Methyl Oleate	C ₁₉ H ₃₆ O ₂	296	55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 41[CH ₂ C=N, Ar] ⁺ 69[C ₅ H ₉ ,CF ₃ ,CH ₃ CH=CHC=O] ⁺ 97[C ₇ H ₃ ,S-Side Chain Ring-CH ₂] ⁺ 98[O-Side Chain Ring-CH ₂ O+H] ⁺
6	2.54	22.205	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺ 41[CH ₂ C=N, Ar] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺
7	0.25	24.480	Methyl elaidate	C ₁₉ H ₃₆ O ₂	296	41[CH ₂ C=N, Ar] ⁺ 55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 69[C ₅ H ₉ ,CF ₃ ,CH ₃ CH=CHC=O] ⁺

						97[C ₇ H ₃ , S-Side Chain Ring-CH ₂] ⁺ 110
8	0.66	24.830	Arachidic acid methyl ester	C ₂₁ H ₄₂ O ₂	326	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺ 41[CH ₂ C=N, Ar] ⁺
9	0.20	27.264	Methyl behenate	C ₂₃ H ₄₆ O ₂	354	55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 41[CH ₂ C=N, Ar] ⁺ 74[CH ₂ CO ₂ CH ₃ +H] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺ 87[C ₃ H ₇ CO ₂] ⁺

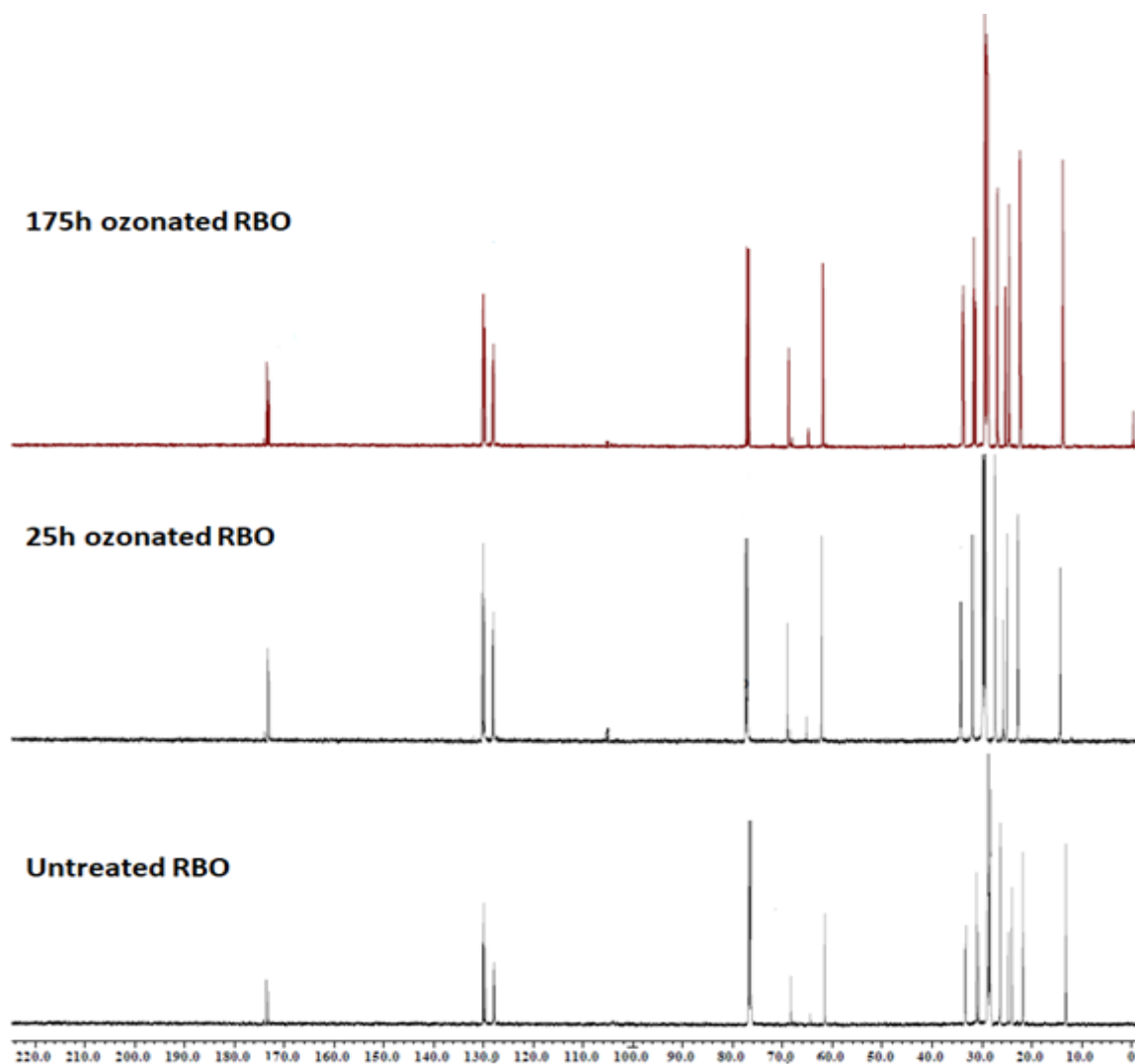


Figure 3. ¹³C NMR Spectra of Untreated and Ozonated RBO

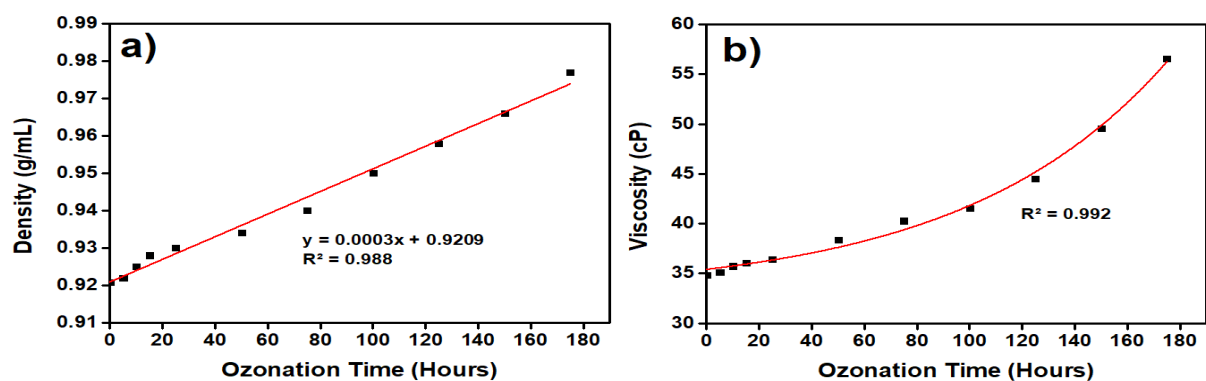


Figure 14. (a) Density and (b) Kinematic Viscosity of RBO during Ozonation Process.

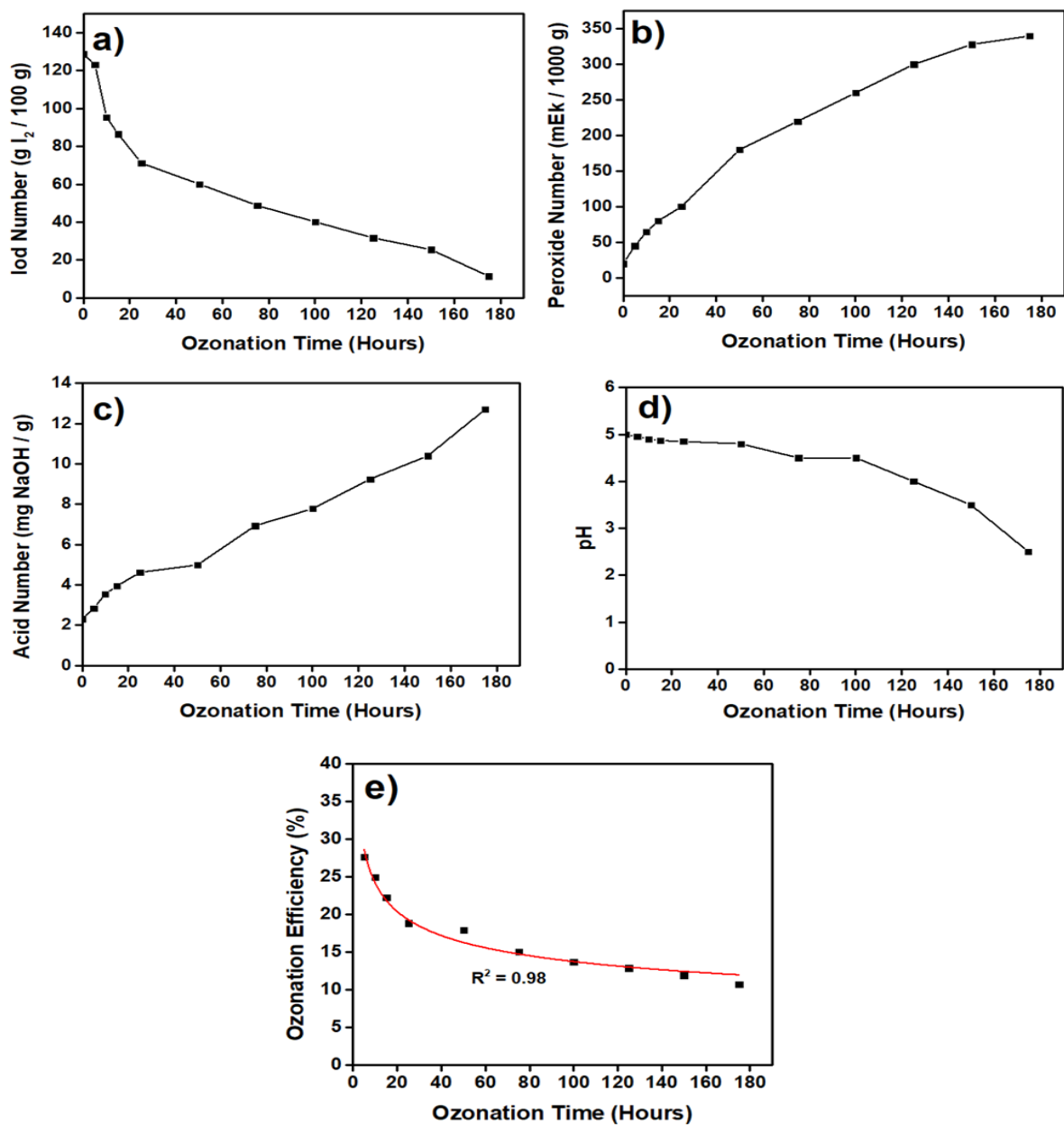


Figure 15.(a) Iodine Number (b) Peroxide Number (c) Acid Number (d) pH and (e) OE of RBO during the Ozonation Process for 175 hour

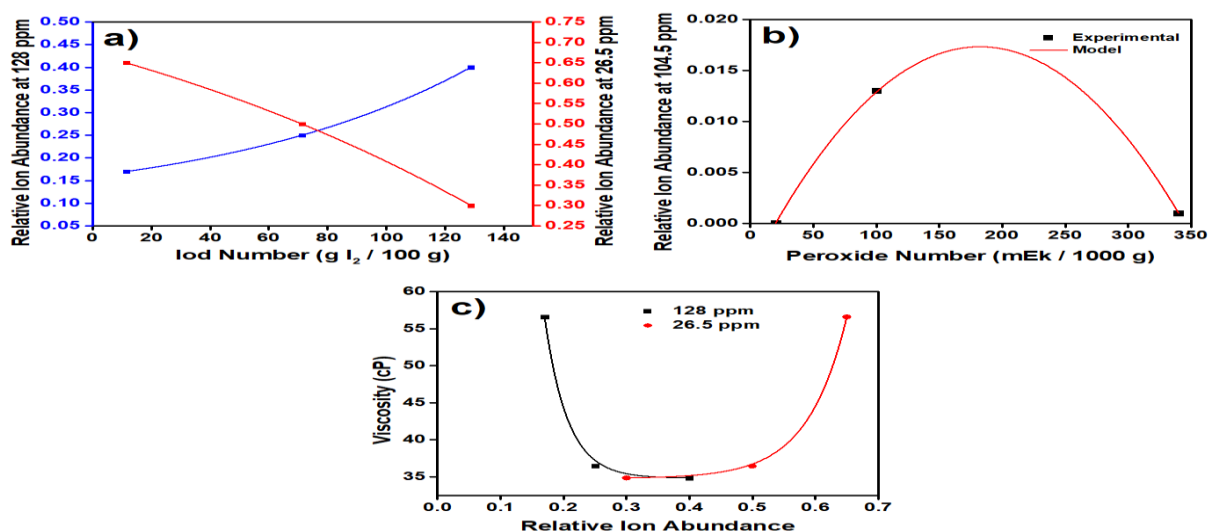


Figure .15 The Characteristic behavior between (a) Alkene and Iodine Number, as well as acyl chains, (b) peroxide number and resulting 1,2,4, Trioxolane and (c)alkene and viscosity, as well as acyl chain

D. STATUS LUARAN: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta unggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui Simlitabmas.

Luaran yang dijanjikan Pada Tahun I adalah :

1. **Luaran Wajib** Jurnal international international accepted (ada 1)
Status: Accepted di jurnal International Journal of Pharmaceutical Research Scopus coverage years:from 2010 to Present Publisher:Advanced Scientific Research ISSN:0975-2366
2. **Luaran Tambahan:** Jurnal international international accepted (ada 1)
Status :
 - a. **Submid** Pada jurnal *Simetrikal Journal of Engineering and Technology* Judul “Rice Bran Oil Extraction as Trioxolane Raw Material”
 - b. **Sudah dilaksanakan di ICB Pharma (International Current Breakthrough in Pharma) UMS Surakarta 16 – 17 Nopember 2019.**

E. PERAN MITRA: Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (untuk Penelitian Terapan, Penelitian Pengembangan, PTUPT, PPUPT serta KRUP). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui Simlitabmas.

F. KENDALA PELAKSANAAN PENELITIAN: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Beberapa Kendala yang dihadapi selama percobaan:

Alat generator yang cocok untuk medis harus dipesan dari China dan memerlukan waktu hampir 2 bulan, kemudian di tambah lagi bahan baku RBO hasil ekstraksi rendement hanya sekitar 18 % sehingga diperlukan waktu lebih lama untuk menunggu bahan dan alat tersebut. Sehingga menyebabkan luaran berupa publikasi tidak tercapai sampai batas waktu ini.

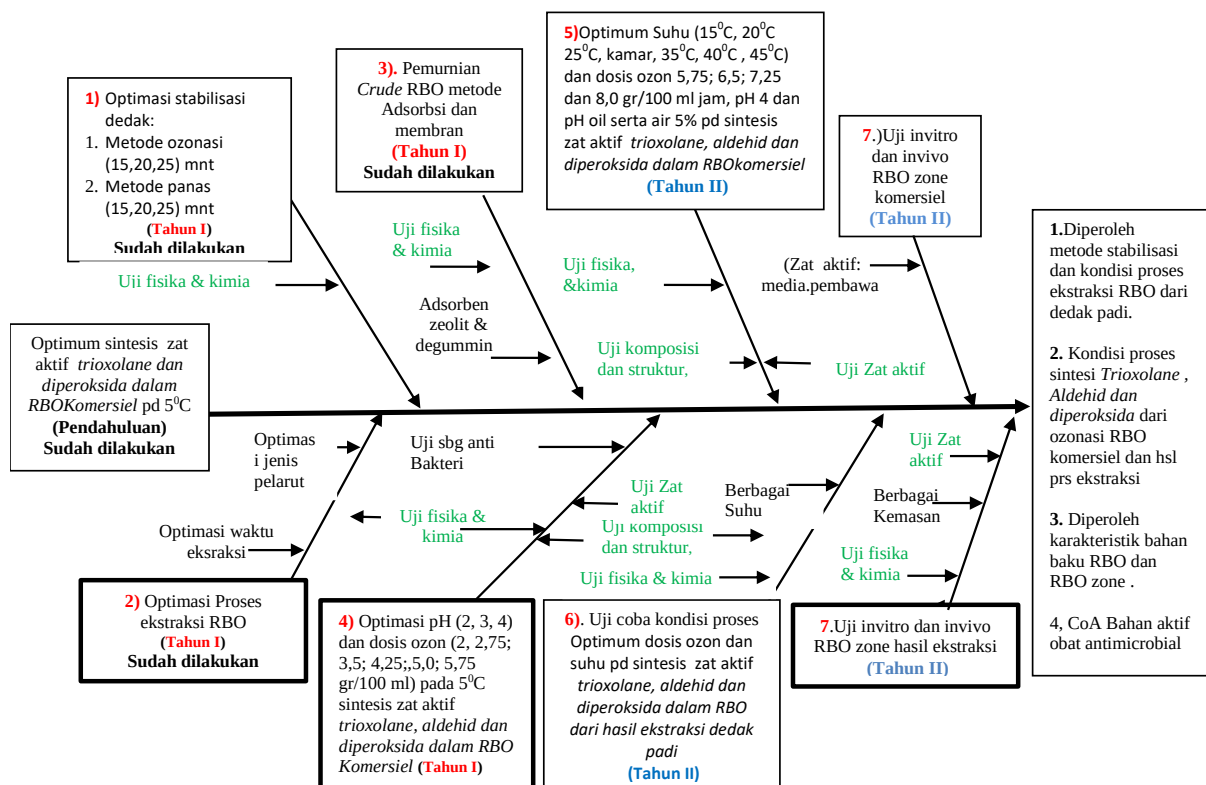
G. RENCANA TAHAPAN SELANJUTNYA: Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Jika laporan kemajuan merupakan laporan pelaksanaan tahun terakhir, pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

Rencana penelitian tahun 2010:

Berdasarkan luaran indikator yang sudah tercapai ternyata proses sintesis zat aktif 1,2,4 Trioxolane, Aldehid dan peroksida berlangsung dengan baik/berhasil, hanya saja dari segi konsentrasi masih kecil. Maka berdasarkan hal tersebut maka direncanakan sbb:

1. Melakukan optimasi suhu proses (15, 20, 25, 30 dan 35 °C) sintesis zat aktif Trioxolane dan diperoksida dalam Rice Brandzone Oil komersiel pada skala 1 liter.
2. Mencoba menggunakan katalis logam untuk mempercepat sintesis zat aktif Trioxolane dan diperoksida dalam Rice Brandzone Oil komersiel pada skala 1 liter.
3. Melakukan uji coba sintesis zat aktif Trioxolane dan diperoksida dalam Rice Brandzone Oil komersiel pada skala 1 liter pada suhu dan katalis optimum pada RBO hasil ekstraksi dedak indonesia.
4. Data hasil uji komposisi Rice Brandzone Oil: zat aktif (Trioxolane, diperoksida dan lainnya), struktur Trioxolane (NMR) dan FT-IR.
5. Melakukan karakteristik RBO hasil ozonasi dan sebelum ozonasi lengkap:
 - a. Uji Fisika (densitas, viscositas, dan warna),
 - b. kimia (Bilangan Asam, peroksida, bilangan Iod dan anti oksidan serta pH),
 - c. Struktur (FTIR dan NMR),
 - d. Komposisi (GCMS)
 - e. Biologi (toksisitas, invitro dan invivo)

Roadmap keseluruhan (selama 2 Tahun):



Penjelasan roadmap, luaran

NO	KEGIATAN	PELAKSANA	INDIKATOR	LUARAN
1.	Optimasi stabilisasi dedak padi dengan metode ozonasi & pemanasan	Tahun I	Metode terbaik ozonasi pada	Jurnal International Judul “Pengaruh Ozonasi stabilisasi Dedak Padi terhadap kualitas RBO yang dihasilkan (sedang disiapkan)
2	1.Optimasi Proses ekstraksi pada berbagai waktu). 2.Pemurnian Crude RBO dengan metode degumming, Adsorpsi dan netralisasi	Tahun I	Diperoleh kondisi proses ekstraksi sudah didapat	Jurnal International Simetrikal Journal of Engineering and Technology Judul “Rice Bran Oil Extraction as Trioxolane Raw Material (Submit)
3	Optimasi pH (2, 3, 4) dan dosis ozon (2, 2,75; 3,5; 4,25; 5,0; 5,75 gr/100 ml) pada 5°C sintesis zat aktif trioxolane, aldehid dan diperoksida dalam RBO Komersiel	Tahun I	Diperoleh pH dan dosis ozon terbaik	Jurnal International: 1. Judul “Spectroscopic and Physicochemical Characteristic of Ozonated Rice Brand Oil as Antimicrobial” (Accepted) 2. Judul “Pengaruh pH dan penambahan Asam Ascorbat pada Karakteristik RBO Zone Oil”
4	Optimum Suhu (15°C, 20°C 25°C, kamar, 35°C, 40°C , 45°C) dan dosis ozon 5,75; 6,5; 7,25 dan 8,0 gr/100 ml jam, pH 4 dan pH oil serta air 5% pd sintesis zat aktif trioxolane, aldehid dan diperoksida dalam RBO komersiel	Tahun II	Diperoleh suhu dan dosis Ozon yang optimal	Publikasi di Jurnal Interbational
5	Uji coba kondisi proses Optimum dosis ozon dan suhu pd sintesis zat aktif trioxolane, aldehid dan diperoksida dalam RBO dari hasil ekstraksi dedak padi	Tahun II	Diperoleh data karakteristik produk	Publikasi di Jurnal Interbational 2 Seminar International
6	Uji invitro dan invivo RBO zone hasil ekstraksi	Tahun II	Diperoleh sifat ssbg anti microbila	Paten
7	Uji invitro dan invivo RBO zone komersiel	Tahun II	Diperoleh sifat ssbg anti microbila	

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan kemajuan yang dicantumkan dalam Daftar Pustaka.

Ali, A., & S. Devarajan. (2017). Nutritional and Health Benefits of Rice Bran Oil. In: Manickavasagan, A., Santhakumar, C., Venkatachalapathy, N. (Eds), *Brown Rice. Springer, Cham*, pp. 135-158. doi :10.1007/978-3-319-59011-0_9

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Spectroscopic and Physicochemical Characteristic of Ozonated Rice Brand Oil as Antimicrobial

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ABSTRACT

Rice Brand Oil (RBO) is a vegetable oil, containing active substances that are very good for health and medicine. The use of RBO can be expanded by ozonation of unsaturated fatty acids in RBO to produce 1,2,4 trioxolane, aldehydes, hydroperoxides and peroxides as anti-viruses in patients with HIV, hepatitis, bird flu etc. The effect of the ozonation process on the characteristics of Rice Bran Oil (RBO) is estimated. Spectroscopic characteristics were analysed by GCMS and ¹³C-NMR, while physical and chemical characteristics were studied by density, viscosity, pH, iodine number, peroxide number, and acid number. In addition, RBO color changes during the ozonation process also change. The carbon double bond in the RBO decreases with increasing ozonation time. 1,2,4 Trioxolane, aldehydes and peroxide are the main products in ozonation reactions. The presence of 1,2,4-trioxolane can be removed by gas chromatography and nuclear magnetic resonance spectroscopy analysis, where it arises during the 25-hour ozonation process, and the expanded field increases and ozonates for 175 hours. From ¹³C-NMR spectrum, the presence of ozonides was confirmed by the signals at δ_c 104.5 ppm. Some saturated fatty acids formed during the ozonation processes from GCMS analysis, such as 1,1-dimethoxydodecane and methyl myristate, proved that ozone as mediator reacted with the unsaturated fatty acid and broke down the double chain of C=C bond to become -C-C- bond. The amount of iodine decreases because the carbon double bonds change into a single bond and the amount of peroxide and acid increases because the component bonds have a single carbon bond, namely 1,2,4 Trioxolane, aldehydes and peroxide. The formation of 1,2,4-trioxolane and other single carbon bond compositions having higher molecular weight increases density and viscosity. Using existing ozone generators requires a longer ozonation time to place the RBO in saturated conditions and make 1, 2, 4-trioxolane unstable and degraded.

Keywords: Ozone, ¹³C NMR, Peroxide Number, Iodine Number, 1,2,4-Trioxolane

INTRODUCTION

Rice Brand Oil (RBO) is a very special vegetable oil, containing active substances which are very good for health and medicines, such as rich in vitamin E (tocopherol and tootrienol), bioactive phytonutrients, phytosterols, γ -oryzanol, squalene alcohol and triterpene (Ali et al., 2017). According to DougDoug (1999), the unsaturated fatty acid content in RBO is around 81,4% which consists of 50,1% oleic acid (C18: 1), 27,1% linoleic acid (C18: 2), and 2.2% linolenic acid (C18: 3), whereas the saturated fatty acids contained in this RBO consist of 0.9% stearic acid and 17.6% palmitic acid. The content of unsaturated fatty acids in RBO is much higher compared to Virgin Coconut Oil (VCO), which is only 6-8% (Ju and Vali, 2005).

The active substances contained in RBO can be as antioxidants (tocopherol and tocotrienol), anti-

inflammatory (Gama-oryzanol), hypocholesterolemia (or-oryzanol), anti-aging (tootrienols) and anticancer properties (Poorna et al, 2016). The benefits of RBO as medicines can be increased through the ozonation process, where carbon-carbon double bonds from unsaturated fatty acids by ozone are converted to molozonida then become 1,2,4 Trioxolane and their stable derivatives such as aldehydes and peroxides (Criegee, 1975) as an anti-microbial (anti-bacterial, fungal and viral). Thus, RBO that has been ozoned has the potential be used to cure patients HIV/AIDS, hepatitis, bird flu and other diseases patients (Herman, 2013). Reaction of the formation of 1,2,4 Trioxolane and its derivatives resulting from the ozonation of unsaturated fatty acids in vegetable oils is presented in Figure 1 (Criegee, 1975).

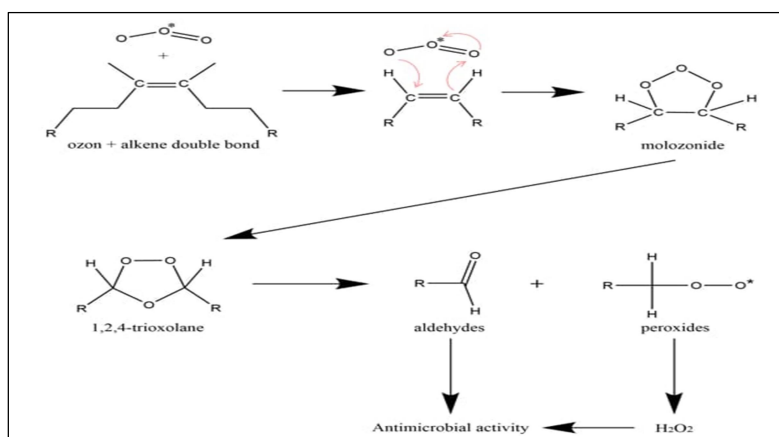


Fig.1: Criegee Mechanism for the Reaction of Ozone with Carbon-carbon Double bonds. (Criegee, R. 1975)

There are many ozonated vegetable oils that have been studied. For instance, Geweely (2006) did research about ozonated olive oil for antifungal properties. Díaz et al., (2005) studied the characteristics of ozonated sunflower oil, while Vallachi et al., (2011) investigated ozonated sesame oil characteristics for skin medicine purpose. Nonetheless, based on our literature searches so far, there are no researchers who have observed or studied ozonated RBO, whereas in fact, ozonated RBO can increase the value of a product, especially in medicine, cosmetics or personal care products, if it is used as one of the ingredients.

Ozonated vegetable oil is produced by an ozonation process from vegetable oil in a certain temperature and ozone dose. Ozonation can occur in unsaturated fatty acids or free fatty acids and produce many important products, such as ozonides or 1,2,4-trioxolane, peroxide and aldehydes (Zanardi et al, 2008; Bailey, 1978). According to Herman (2008), 1,2,4-trioxolane in ozonated vegetable oil can be used as an active ingredient for the treatment of diseases caused by microorganisms (antibacterial), fungi (anti fungicidal), and viruses (antivirus). So, it is suitable to be applied as an ingredient in the pharmaceutical and cosmetic industries. The content of ozonide or 1,2,4-trioxolane in ozonated vegetables oil is very dependent on process conditions, such as temperature, ozone dose, and the form of reactor used (Zanardi et al, 2008).

In this study, RBO was treated with an ozonation process to produce ozonated RBO, which has good properties for pharmaceutical or cosmetic needs. Spectroscopic characteristics were established by analyzing the RBO (before and

after ozonation) using gas chromatography–mass spectrometry (GC-MS) and Nuclear magnetic resonance (NMR) spectroscopy. Physicochemical characteristics were obtained by analyzing kinematic viscosity, density, iodine number, peroxide number and acid number of RBO. Correlation between spectroscopy and physicochemical characteristic were also studied.

MATERIALS AND METHODS

Materials

Chemicals such as ethanol, phenolphthalein, NaOH, acetic acid glacial, chloroform, potassium iodide, sodium thiosulphate, were purchased from Merck and used without further purification. Starch and Rice Bran Oil were obtained from a local market in South Tangerang, Indonesia. The physicochemical characteristics of RBO can be shown in Table 1.

The Method Of Ozonation

The ozonated RBO was produced by a method described by Zanardi et al., (2008). Briefly, ozone/oxygen mixture was bubbled in a jacketed bubble column reactor containing 1000 mL of RBO for different times comprised between 5 and 175 hours (i.e. 5, 10, 15, 25, 50, 75, 125, 150, 175 hours). The operating temperature was kept at 5 °C and the RBO didn't freeze. Ozone was produced through a RS 09805 (60/50HZ) 110/220 Volt from ozone procesor Resson (China) with dengan output ozon sebesar 0,025 g. L⁻¹ per hour. The off-gas which contained non-reacted ozone was directed to two absorption containers, containing 100 ml acetic acid glacial and potassium iodide. A stirrer with a rotation rate of 750 rpm was set inside the reactor to make the RBO more homogenous. The ozonated RBO was then collected from the reactor to be

analyzed using NMR and GCMS, as well as for its physicochemical characteristic

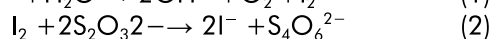
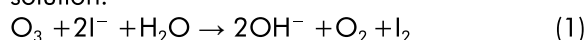
NMR and GCMS analysis

The ^{13}C NMR spectra were collected from a JEOL JNMEX400 and JNM ECA500 single pulse spectrometer at 25 °C on untreated RBO and on RBO after 25 h, and 175 h of ozone treatment in CDCl_3 . All the experiments were performed under the same experimental conditions and concentrations. The ^{13}C spectra were recorded with a relaxation delay of 2 s; a total of 1021 scans were collected for each sample with a 30° excitation pulse.

GCMS analysis of the untreated RBO and on RBO after 25 h, and 175 h of ozone treatment was performed using Shimadzu GCMS-QP2010 (Kyoto, Japan). Column temperature was programmed from 70-230°C at increasing rate of 10°C/min, held at initial for 1 min and equilibrium time for 3 min. The major peaks were analyzed by comparing its mass fragments patterns with standard spectra available in NIST library.

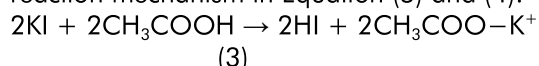
Iodine Number

Iodometric titration was used in this experiment to calculate the amount of ozone consumed by the oils/fatty acids. The reaction between iodide and ozone resulted in free iodine as shown in Equation 1. Then iodine was reacted with sodium thiosulphate, and starch acted as an indicator as shown in Equation 2. As an indication of equivalence point, the color would change from purple to colorless (Sadowska et al., 2008). The De-Ionized (DI) water was used as a blank solution.



Peroxide Number

According to British Pharmacopoeia, (2000a), Peroxide Number (PN) is the number which expresses the quantity of peroxide, in milli equivalents of active oxygen, which is contained in 1000 g of the substance. The PV of both untreated and ozonated samples were determined using the American Oil Chemists' Society (AOCS) official method, following the reaction mechanism in Equation (3) and (4):



Peroxide ($\text{R}\cdot\text{OO}\cdot\text{H}$) will react with potassium iodide in the presence of acetic acid to result in iodine. Then, iodine is titrated with a sodium thiosulfate solution following Equation (2).

Acid Number

Acid Number (AN) according to British Pharmacopoeia, (2000b), is the number of base mass required (in mg) to neutralize the free acids per gram of the substance. Moreover, the acid value shows the degree to which the triglycerides in the oil sample have broken down to form free fatty acids. Sodium hydroxide was used as titrant to titrate the mixture solution between the oil sample and ethanol, while phenolphthalein acted as an indicator (Almeida Kogawa et al., 2015).

Ozonation Efficiency

The Ozonation Efficiency (OE) can be described as the ratio between the amount of peroxidation due to the ozonation process and the amount of total ozone applied to the system (Sega et al., 2010). It was calculated with Equation (8):

$$OE = \frac{PN_s - PN_0}{1000} \times \frac{mEq \text{ O}_3}{AOD} \times 100\% \quad (8)$$

where PN_s and PN_0 are the Peroxide Number of the ozonated and untreated RBO, respectively; $mEq \text{ O}_3$ is the ozone equivalent weight; and AOD represents the applied ozone dose (in mg g^{-1}).

Density, Kinematic Viscosity, and pH analysis

The density measurements were done by measuring the weight of pycnometer in the absence and presence of RBO samples. The kinematic viscosity was measured with Ostwald capillary viscometers at 25 °C, and pH was measured using a digital pH meter

RESULTS AND DISCUSSION

Spectroscopic Characterization

GCMS analysis

The GCMS analysis of untreated and ozonated RBO after 175 h is showed in Figure 2. There are two noticeable things found in that figure. First, the six peaks of spectra found in the GCMS analysis of untreated RBO indicating palmitic acid, methyl linoleate, methyl oleate, methyl stearate, montanic acid, and arachidic acid, with the highest spectra being methyl oleate. Fragmentation patterns were obtained from a spectrogram of each component, where there were special basic peaks in each fragmentation pattern (Silverstein, 2005). Second, nine peaks appeared in the GCMS analysis of RBO after 175h of ozonation process. The greater number of peaks show that some reaction occurred inside the RBO during ozonation process. The nine peaks in ozonated RBO refer to 1,1-Dimethoxydodecane, methyl myristate, palmitic acid, methyl linoleate, methyl oleate, methyl

stearate, methyl elaidate, arachidic acid methyl ester, and methyl behenate. These components are more or less the same as most components

found in other vegetable oils. The methyl oleate still has the highest spectra compared to other peaks although the ozonation process occurred.

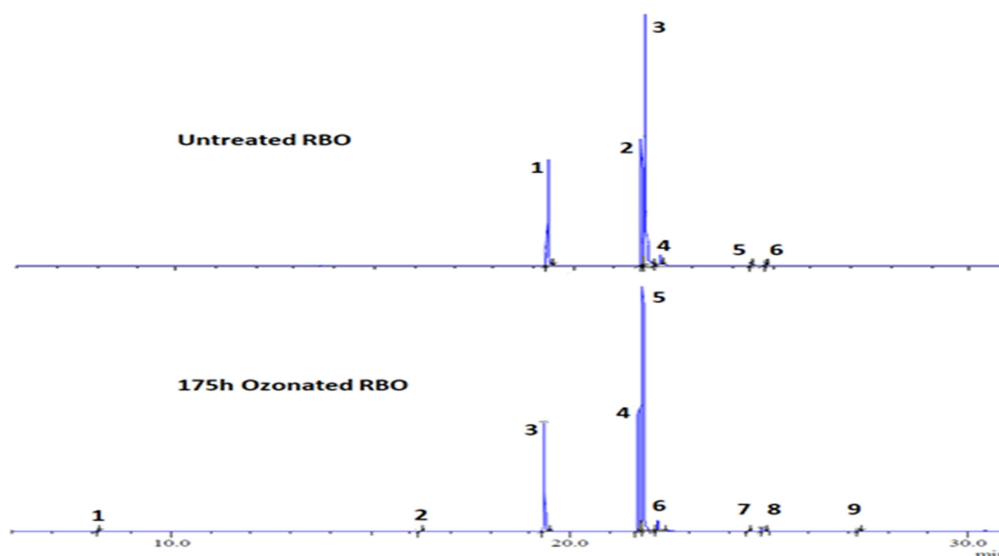


Fig.2: The GCMS analysis of untreated and ozonated RBO after 175 h

The complete data of retention time, area percentage, and ion mass from untreated and ozonated RBO is showed in Table 2 and Table 3. From the comparison between the chemical components in untreated and ozonated RBO, some saturated fatty acids formed during the ozonation processes, such as 1,1-Dimethoxydodecane, methyl myristate, and methyl behenate. It proves that ozone as mediator reacted with the unsaturated fatty acid and broke

down the double chain of C=C bond to become -C-C- bond. Moreover, -C-C- bond bound with oxygen to form O=C-C=O as saturated fatty acid. Unfortunately, the cyclic 1,2,4-trioxolane couldn't be found because of its unstable nature (Almeida, 2012). This instability made trioxolane difficult to detect in GCMS. Another characterization such as NMR can reveal the presence of trioxolane in ozonated RBO.

Table 1: Physicochemical Characteristics of Rice Bran Oil as Raw Material

Parameter	Result	Unit
Color	Yellowish	-
Odor	Objectional	-
Density	0.921	g/mL
Kinematic viscosity	34.85	cP
pH	5.0	-
Iod Number	128	g I ₂ /100 g
Acid Number	2.311	mg NaOH/g
Peroxide Number	20	mEk/1000 g

Table 2: Chemical component of untreated RBO showed from GCMS analysis

Peak No.	%Area	Retention Time	Molecule name	Formula	Molar mass	Ion Mass (m/z)
1	20.26	19.351	Palmitic acid	C ₁₇ H ₃₄ O ₂	270	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺

2	23.51	21.724	Methyl linoleate	$C_{19}H_{34}O_2$	294	$41[CH_2C=N, Ar]^+$ $57[C_4H_9, C_2H_5C=O]^+$ $55[C_4H_7, CH_2=CHC=O]^+$ $67[C_5H_7]^+$ $81[Side\ Chain\ Ring-CH_2, C_6H_9]^+$ $41[CH_2C=N, Ar]^+$ $95[Side\ Chain\ Ring-C=O]^+$
3	53.62	21.827	Methyl Oleate	$C_{19}H_{36}O_2$	296	$55[C_4H_7, CH_2=CHC=O]^+$ $41[CH_2C=N, Ar]^+$ $69[C_5H_9, CF_3, CH_3CH=CHC=O]^+$ $97[C_7H_3, S-Side\ Chain\ Ring-CH_2]^+$ $98[O-Side\ Chain\ Ring-CH_2O+H]^+$
4	2.03	22.204	Methyl stearate	$C_{19}H_{38}O_2$	530	$41[CH_2C=N, Ar]^+$ $69[C_5H_9, CF_3, CH_3CH=CHC=O]^+$ $83[C_6H_{11}, CH^{35}Cl_2]^+$ $96[(CH_2)_5\equiv N]^+$ $111[S-Side\ Chain\ Ring-C=O]^+$
5	0.19	24.492	Octacosanoic acid	$C_{29}H_{58}O_2$	530	$41[CH_2C=N, Ar]^+$ $69[C_5H_9, CF_3, CH_3CH=CHC=O]^+$ $83[C_6H_{11}, CH^{35}Cl_2]^+$ $96[(CH_2)_5\equiv N]^+$ $111[S-Side\ Chain\ Ring-C=O]^+$
6	0.39	24.828	Arachidic acid methyl ester	$C_{21}H_{42}O_2$	490	$74[CH_2CO_2CH_3+H]^+$ $87[C_3H_7CO_2]^+$ $43[C_3H_7, CH_3C=O, C_2H_5N]^+$ $41[CH_2C=N, Ar]^+$ $57[C_4H_9, C_2H_5C=O]^+$

Table 3: Chemical Component of Ozonated RBO Shown from GCMS Analysis

Peak No.	%Area	Retention Time	Molecule Name	Formula	Molar mass	Ion Mass (m/z)*
1	0.23	8.138	1,1-Dimethoxydodecane	$C_{14}H_{30}O_2$	230	$71[C_5H_{11}, C_3H_7C=O]$ $41[CH_2C=N, Ar]^+$ $55[C_4H_7, CH_2=CHC=O]^+$ $58[CH_3C(=O)CH_2+H, C_2H_5CHNH_2]$ $96[(CH_2)_5\equiv N]^+$
2	0.13	16.249	Methyl myristate	$C_{15}H_{30}O_2$	242	$74[CH_2CO_2CH_3+H]^+$ $87[C_3H_7CO_2]^+$ $41[CH_2C=N, Ar]^+$ $57[C_4H_9, C_2H_5C=O]^+$
3	20.22	19.356	Palmitic acid	$C_{17}H_{34}O_2$	270	$74[CH_2CO_2CH_3+H]^+$ $87[C_3H_7CO_2]^+$ $43[C_3H_7, CH_3C=O, C_2H_5N]^+$ $41[CH_2C=N, Ar]^+$ $57[C_4H_9, C_2H_5C=O]^+$
4	23.8	21.730	Methyl linoleate	$C_{19}H_{34}O_2$	294	

0				2		55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 67[C ₅ H ₇] ⁺ 41[CH ₂ C=N, Ar] ⁺ 81[Side Chain Ring-CH ₂ ,C ₆ H ₉] ⁺ 55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 109[Side Chain Ring-C=O]
5	51.9 7	21.833	Methyl Oleate	C ₁₉ H ₃₆ O 2	296	55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 41[CH ₂ C=N, Ar] ⁺ 69[C ₅ H ₉ ,CF ₃ ,CH ₃ CH=CHC=O] ⁺ 97[C ₇ H ₃ ,S-Side Chain Ring-CH ₂] ⁺ 98[O-Side Chain Ring-CH ₂ O+H] ⁺
6	2.54	22.205	Methyl stearate	C ₁₉ H ₃₈ O 2	298	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺ 41[CH ₂ C=N, Ar] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺
7	0.25	24.480	Methyl elaidate	C ₁₉ H ₃₆ O 2	296	41[CH ₂ C=N, Ar] ⁺ 55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 69[C ₅ H ₉ ,CF ₃ , CH ₃ CH=CHC=O] ⁺ 97[C ₇ H ₃ , S-Side Chain Ring-CH ₂] ⁺ 110
8	0.66	24.830	Arachidic acid methyl ester	C ₂₁ H ₄₂ O 2	326	74[CH ₂ CO ₂ CH ₃ +H] ⁺ 43[C ₃ H ₇ ,CH ₃ C=O,C ₂ H ₅ N] ⁺ 87[C ₃ H ₇ CO ₂] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺ 41[CH ₂ C=N, Ar] ⁺
9	0.20	27.264	Methyl behenate	C ₂₃ H ₄₆ O 2	354	55[C ₄ H ₇ ,CH ₂ =CHC=O] ⁺ 41[CH ₂ C=N, Ar] ⁺ 74[CH ₂ CO ₂ CH ₃ +H] ⁺ 57[C ₄ H ₉ ,C ₂ H ₅ C=O] ⁺ 87[C ₃ H ₇ CO ₂] ⁺

¹³C NMR Analysis

Figure 3 shows the NMR spectra of untreated and ozonated RBO and assignments to all pertinent peaks were summarize in Table 4. NMR analyses further confirmed the structural changes undergone by untreated RBO methyl esters during ozonation. New signal at 104.5 ppm was assigned to the ring carbon of 1,2,4-trioxolane. The signal with δ = 130.139 ppm is the chemical shift of oleic acid, while the signal with δ =

128.022 ppm is a chemical shift of linoleic acid. From the picture it can be seen that the spectrum of oleic and linoleic acids signals is high; this indicates that the portion of the C=C double bond found in oleic and linoleic acids is also high. The presence of peaks between 10–50 ppm was attributed to sp³-hybridized carbons which indicates that untreated RBO dominantly contains saturated hydrocarbons (lorio et al., 2016).

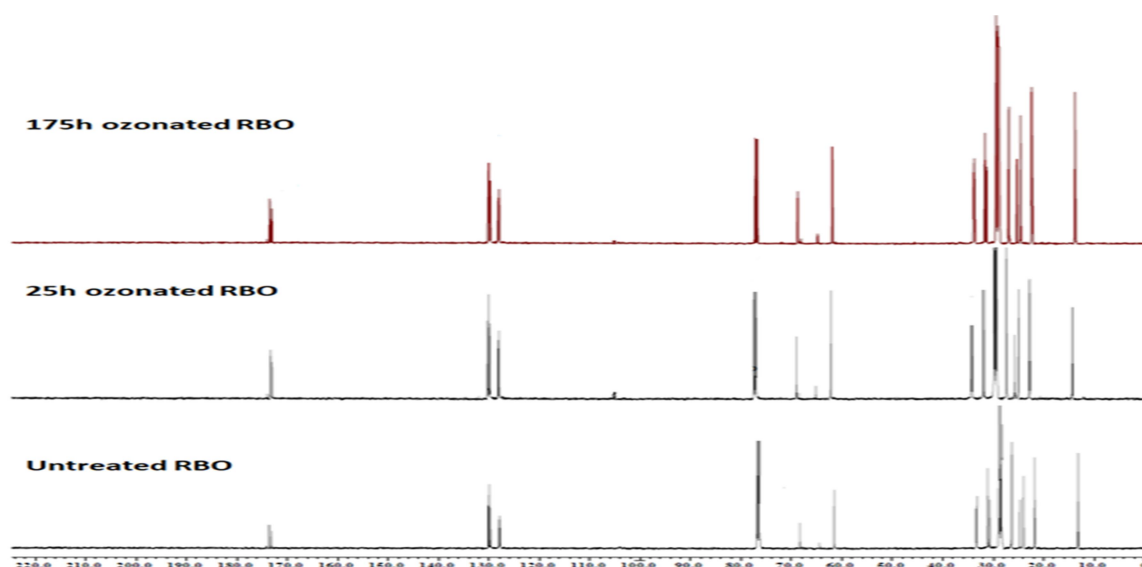


Fig.3: Full ^{13}C NMR Spectra of 175, 25 h Ozonated RBO and Untreated RBO

There were 10 primary signals found in the ozonated RBO after 25 h of ozonation process. In the process of ozonation, the reaction that occurred was an addition of ozone to double bonds in unsaturated fatty acids (oleic and linoleic). The ozonolytic reaction breaks the double bonds in oleic and linoleic acids and replaces them with O bonds to form cyclic -C-O-bond, so that a new ozonide compound is formed, namely 1,2,4-trioxolane (Soriano et al., 2003). The existence of 1,2,4-trioxolane in ozonated RBO can be seen in the new signals that appear on the spectrum, with $\delta = 104,5078$ ppm. Unfortunately, 1,2,4-trioxolane peak disappeared when RBO was ozonated for 175 h. This shows that long-time ozonation process made the solution become saturated, and then 1,2,4-trioxolane degraded into aldehyde form and peroxide, due to its unstable nature, coherent with above mentioned GCMS data in Figure 1. The breakdown of unsaturated fatty acid by ozone is proved by a significant decrease in the intensity of the signals from oleic and linoleic acid in $\delta = 130$ and 128 ppm, respectively. The other

evidence of ozone attack on C double bonds is an increasing signal in chemical shifts around 24 and 31 which are related to methylene protons α and β to ring carbons, respectively (Sega et al., 2010). The formation of ozonides and the disappearance of unsaturated fatty acids are almost equal. The trioxolane ring is relatively stable since the proton signals from the aldehyde group at around 200 ppm are not visible in NMR spectra at each stage of the ozonation process. Some signal in chemical shift of $\delta = 69.018$ - 69.056 , 65.174 - 65.210 , and 62.227 - 62.263 had interesting behaviour, which is related to triglycerides and the alcohol chain. The behaviour showed that the intensity of the signal increased until a certain time, then decreased. It is related to the trioxolane production from ozone oxidation reaction with unsaturated fatty acids which is unstable (Almeida et al., 2013). The complete ^{13}C NMR chemical data of RBO before and after ozonation process for 25 and 175 hours can be shown in Table 4.

Table 4 : ^{13}C NMR data of RBO during ozonation process for 25 and 175 hours

Formula	Bonding name	Chemical shift (ppm)			Behavior
		untreated RBO	25 h ozonated RBO	175 h ozonated RBO	
C=C	Alkenes	130,139	130,185	130.192	decreasing
C=C	Alkenes	128,022	128,048	128.064	decreasing
ROOC-	Carboxyl	173,377	173,451	173.492	increasing
CH ₂ -OCOR	Triglycerides	69,018	69,035	69.056	increasing, then decrease
RCH ₂ O-	Alcohol	65,174	65,210	65.302	increasing, then

CH ₂ - OCOR	Triglycerides	62,227	62,263	62.289	decrease increasing, then decrease
-(CH ₂) ₂ -	Acyl chains	31.021	31.045	31.057	increasing
-(CH ₂) ₂ -	Acyl chains	29,815	29,842	29.881	increasing
RH ₂ COOH	Acyl chains	26.515	26.532	26.548	increasing
RCH ₃	Acyl chains	14,220	14,256	14.277	increasing
C ₂ H ₄ O ₃	1,2,4-trioxolane	-	104,508	-	increasing, then decrease
CDCl ₃	Deuteratedchloroform	77.459	77.448	77.455	stable

Physicochemical Characterization

Density and Viscosity

Figure 4a-b shows the density and kinematic viscosity of ozonated RBO for various ozonation times. The density of ozonated RBO increased linearly as ozonation time increased, with a determination coefficient of 0.988. The formation of new molecules with a higher molar mass causes the density of ozonated RBO to increase (Uysal, 2014). This indirectly proves that the ozone broke down the double bond and produced 1,2,4-trioxolane, which has a relatively

large molar mass (Criegee, 1975). An increase of the ozonation time also led to an exponential trend in the increase of ozonated RBO viscosity. Ozonated RBO reached viscosity values up to 56.59 cP under room temperature, or 65.47% from its initial value, after 175 h of ozonation time. These phenomena match the experiment conducted by Skalka et al., (2009). The increased viscosity for ozonated RBO indicates that the carbon double bonds reacted with ozone to form carbon single bond species.

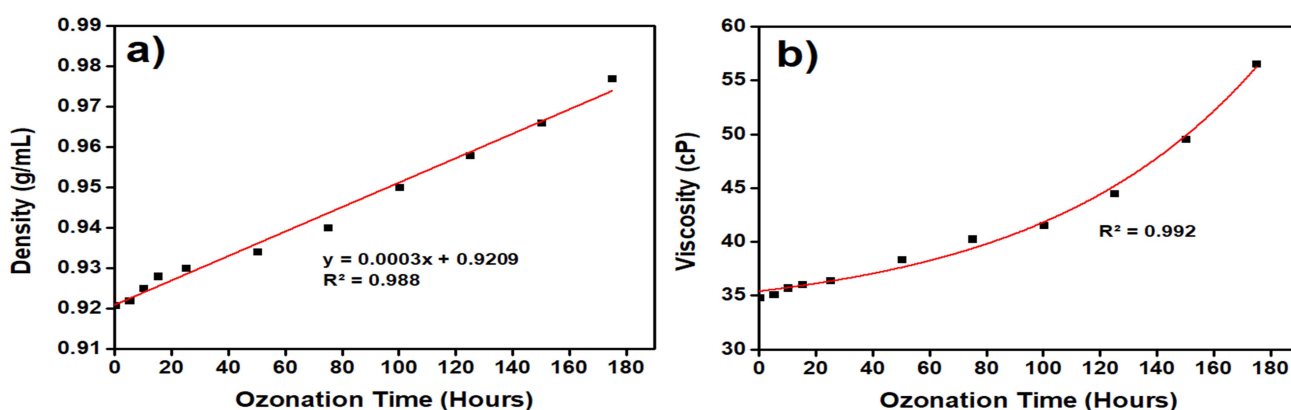


Fig.4: (a) Density and (b) Kinematic Viscosity of RBO during Ozonation Process.

Iodine, Peroxide, and Acid Number analysis

The analysis of iodine numbers in ozonated RBO aims to show a decrease in the amount of iodine with increasing ozonation time as shown in Figure 4a. Iodine numbers for ozonated RBO after 175 h of ozonation time decreased up to 91.14% from their initial value. The iodine numbers decreased rapidly during the first 25 h of ozonation time and then decreased slightly until the end of 175 h. This shows that almost all the double bonds in unsaturated fatty acids react with ozone as a strong oxidative agent, or in other words a decrease in the degree of unsaturation has occurred (Wasmi et al., 2015). The reaction with ozone leads to the gradual disappearance of the

double bond, in line with the result of ¹³C NMR in the previous section.

The Peroxide Number is the most important analysis in ozonated oil since it indicates the amount of peroxide compounds formed. The Peroxide number increases almost linearly with the increase of ozonation time, and reaches saturated condition after a certain time, as shown in Figure 4b. In these phenomena, the partial reaction of ozone in the RBO compartment is evidenced by the increase in the amount of ozone recovered in the iodide trap. This occurs if we assume that most of the unsaturated substance portion becomes slow to react with a certain time, due to probabilistic and steric conditions (Razumovskii and Zaikov, 1980). The formation

of polymeric peroxide responsible for viscous mass is achieved after a massive ozonation process. The Peroxide number for ozonated RBO with the ozonation time of 175 h was up to 340 mEk per 1000 g of RBO. The formation of

peroxidic compounds, especially 1,2,4-trioxolane, is usually correlated by increasing peroxide numbers. Increasing the peroxide number can also be caused by the ozone changes to free oxygen (O_2).

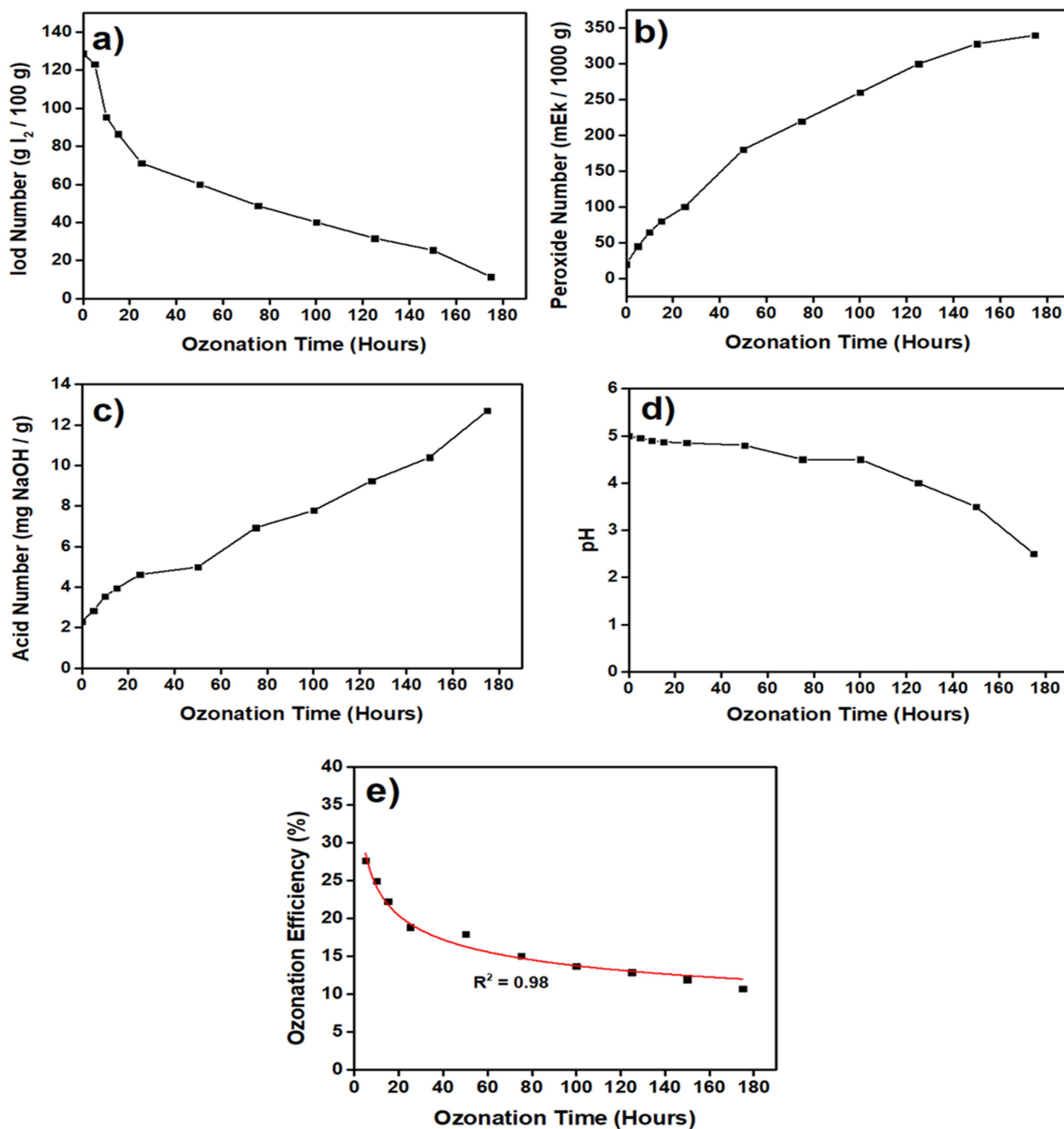


Fig.5(a) Iodine Number (b) Peroxide Number (c) Acid Number (d) pH and (e) OE of RBO during the Ozonation Process for 175 hour

Another parameter to characterize ozonated RBO is an Acid Number. The acid number increases with the increase of ozonation time, as shown in Figure 5c. Acid numbers are usually used to assess the freshness of an oil, and if stored properly. The success of the ozonization process is indicated by an increase in the acid number. During the ozonation process, partial hydrolysis occurs. and acid reaction occurs between

unbound fatty acids and fat, even though fat shows a neutral reaction (Skalka et al., 2009). The decrease in pH of ozonated RBO has a correlation with the increase in acid number (Figure 5d). It is clear because as the acid number increased, the amount of proton in the ozonated RBO also increased. The increasing proton made the pH of the solution decrease. There was a phenomenon related to the pH of ozonated RBO,

where the pH decreased insignificantly until ozonation time achieved 100 hours from 5 to 4.5. After that, the pH decreased significantly from 4.5 to 2.5. According to Langlais (1991), the ozonation effect is faster if the pH of the solution is low, so the profile of pH decrease in the low pH range gives a more significant decrease. Ozone is more stable at low pH so that the amount of ozone that reacts with double bonds in unsaturated fatty acids is more and then produces carboxylic acid.

OE represents the estimated number of ozonated compounds inside RBO, the same as described by the peroxide number, with the amount of ozone used. As expected, OE results decreased because of the disappearance of double bonds as time

increased (longer ozonation period) and its R^2 value was 0.98.

The Color of Ozonated RBO

The color of RBO changed after the ozonation process from clear yellow to dark yellow. The yellow color pigment in RBO comes from oil-soluble carotenoids (Orthoefer and Eastman, 2005). The change in the color of oil after ozonation is because carotenoid pigments (α and β carotene) are oxidized to new carotenoid compounds with lower molecular weights (Rodriguez-Amaya, 2001). This color change is also caused by the oxidation process of ozone to carbon groups in the double bonds of unsaturated fatty acids found in the tocopherols contained in the RBO (Schwartz et al., 2008).



Fig.6: The Colour RBO During (a) 0 (b) 25 hour and (c) 175 hours ozonation time

Correlation between NMR spectra and physicochemical characteristic

The iodine number and peroxide number are more important characteristics compared to the acidic number and pH. Their correlation with NMR peaks is very important to study. In Figure 7a, as the iodine number increased the relative ion abundance value of 128 ppm (alkenes) also

increased, whereas the relative ion abundance of 26.5 ppm (acyl chains) decreased. These phenomena are in agreement with the explanation of Figure 3, Figure 5a, and Table 4. With increasing ozonation time, the carbon double bond is broken by ozone to become a single bond.

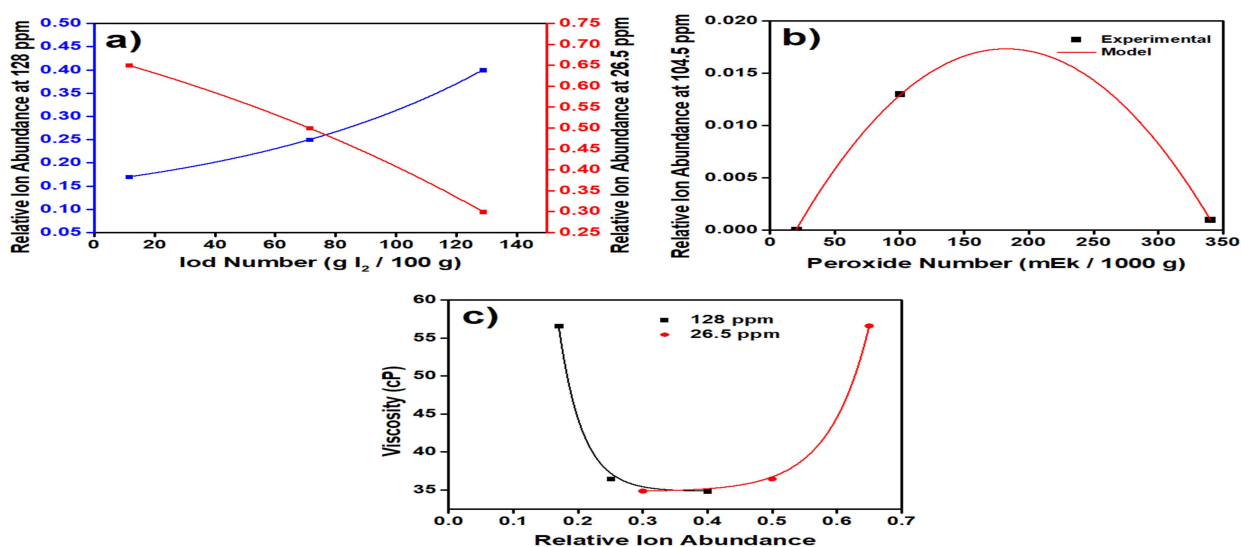


Fig.7: The Characteristic behavior between (a) Alkene and Iodine Number, as well as acyl chains, (b) peroxide number and resulting 1,2,4, Trioxolane and (c)alkene and viscosity, as well as acyl chain

Figure 7b shows that the relative ion abundance value at 104.5 ppm (1,2,4-trioxolane) increased until a certain point before finally coming down, as the the peroxide number increased. The breakdown of carbon double bonds into single bonds resulted in a new valuable component inside RBO, i.e. 1,2,4-trioxolane. But, the long ozonation process made the system of ozonated RBO become saturated with ozone, and also caused the 1,2,4-trioxolane to become unstable, decrease, and then disappear. These phenomena are in harmony in Figure 1, Figure 2, and Figure 4b.

CONCLUSIONS

Ozonolysis of RBO was suggested to follow the Criegee mechanism. The formation of 1,2,4-trioxolane was seen in the RBO after ozonation process at 25 h and 175 h. The density and viscosity of ozonated RBO increases due to the breakdown of carbon double bonds by ozone to become carbon single bonds, which have relatively higher molecular weight. The decrease in iodine number by as much as 54.84%, from 3.748 to 1.693 g I₂ per 100 g of sample, during the ozonation process of 175 hours shows that most of the double bonds in saturated fatty acids react with ozone. Increased peroxide value is observed where the value increases 16-fold during the 175 hours ozonation process. Moreover, the acid number increases 5.5-fold from 2.311 to 12.712 mg NaOH per g of sample. The maximum OE is 27.66% in the first 5 hours of ozonation and then decreases up to 10.72% after 175 hours of ozonation process.

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The correlation between viscosity and carbon double or single bonds is shown in Figure 6c. As the number of carbon double bonds or unsaturated fatty acid increased, the viscosity of RBO decreased. The decrease in viscosity occurred because there was no high molecular weight species in the RBO. This was confirmed by the increase in relative ion abundance of acyl chains, which are carbon single bonds that resulted from the ozonation breakdown process of carbon double bonds.

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Effect of pH and Ozone Dosage on Characteristic of Ozonated Rice Bran Oil**E. Enjarlis^{1*}, Marcelinus Christwardana², Sri Handayani¹, Sofa Fajriah², Setijo Bismo³, Jehuda Reinhard Rahmani¹, Muhammad Tama Hazadin¹**¹ Department of Chemical Engineering, Institut Teknologi Indonesia, Jl. Raya Puspiptek Serpong, South Tangerang, Banten, 15314 Indonesia² Department of Chemistry, Diponegoro University, Jl. Prof. Sudarto SH, Tembalang, Semarang, Central Java, 50275 Indonesia³ Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency, Kawasan PUSPIPTEK, Serpong, South Tangerang, Banten, 15314 Indonesia⁴ Department of Chemical Engineering, Universitas Indonesia, Kampus Baru UI Depok, Depok, West Java, 16424 Indonesia*Corresponding author email: enjarlis@gmail.com**Received** March 01, 2022; **Accepted** August 31, 2022; **Available online** November 20, 2022

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.

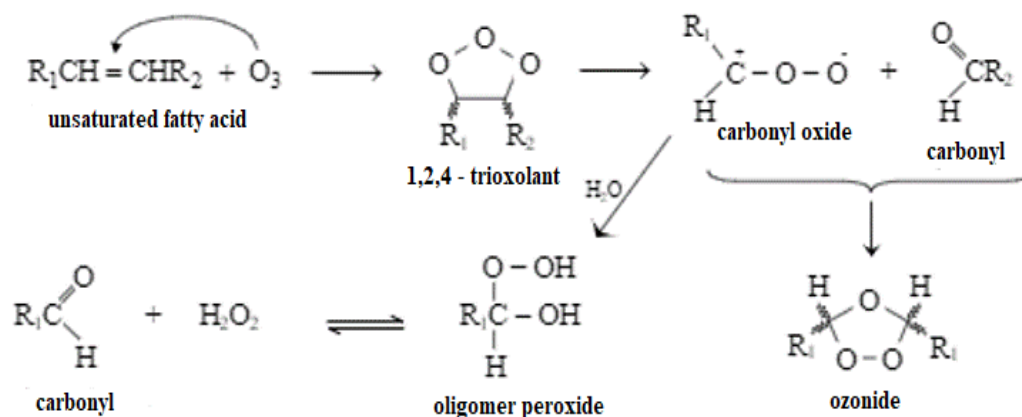


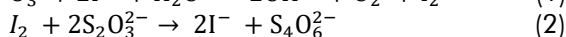
Figure 1. Ozonation mechanism in unsaturated fatty acid

Table 1. Characteristic of RBO before ozonation process

Composition	Value	Unit
Iodine Number	147.204	g Iodine/100 g Oil
Peroxide Number	5	$\text{mg}_{\text{eq}}/\text{kg}$ 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

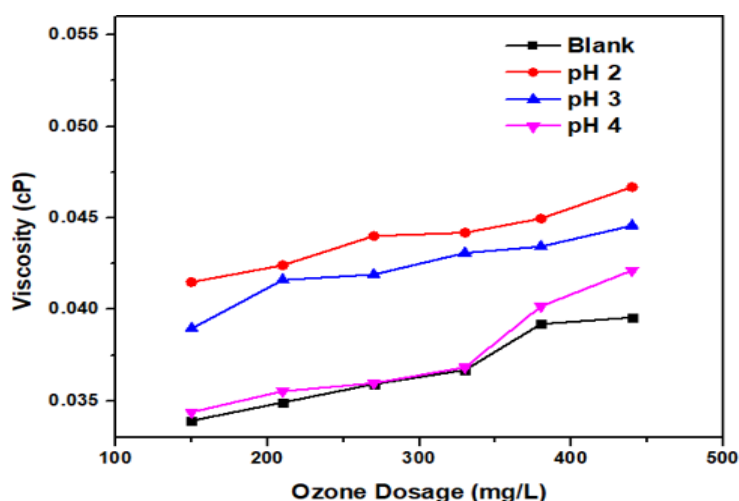


Figure 2. The Effect of pH and Ozone Dosage on Viscosity of Ozonated RBO

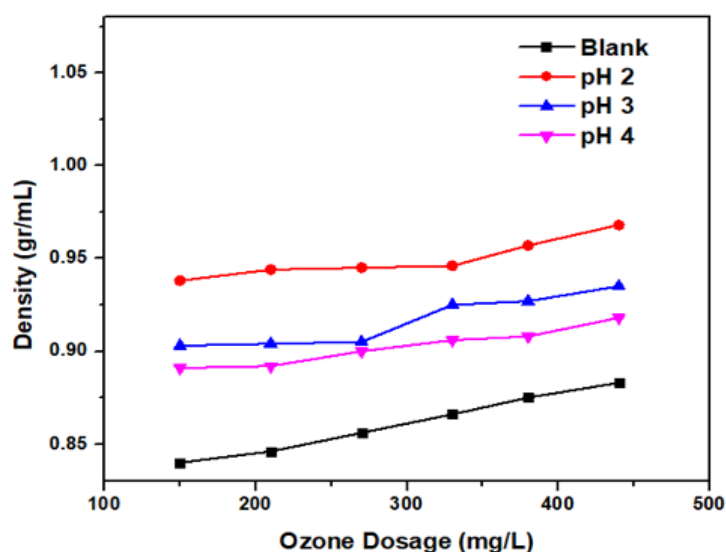


Figure 3. The Effect of pH and Ozone Dosage on Density of Ozonated RBO

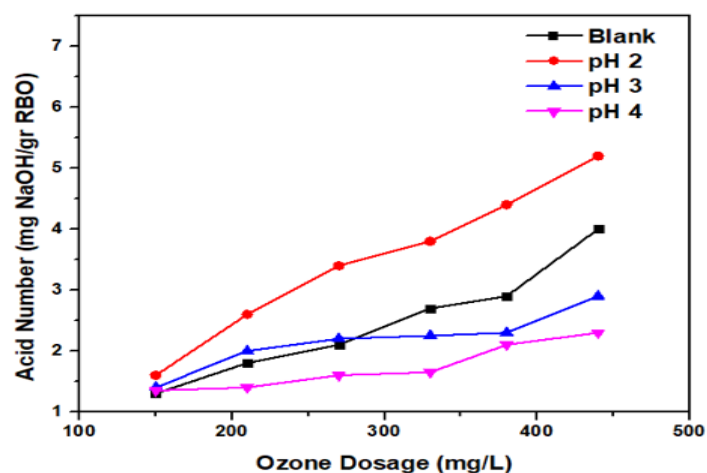


Figure 4. The Effect of pH and Ozone Dosage on Acid Number of Ozonated RBO

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).

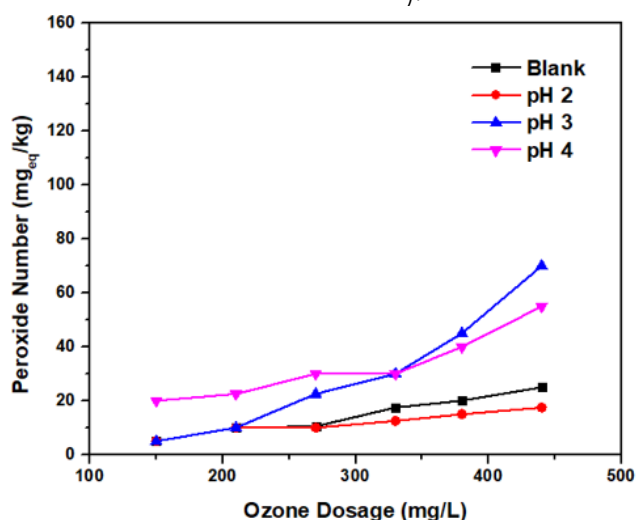


Figure 5. The Effect of pH and Ozone Dosage on Peroxide Number of Ozonated RBO

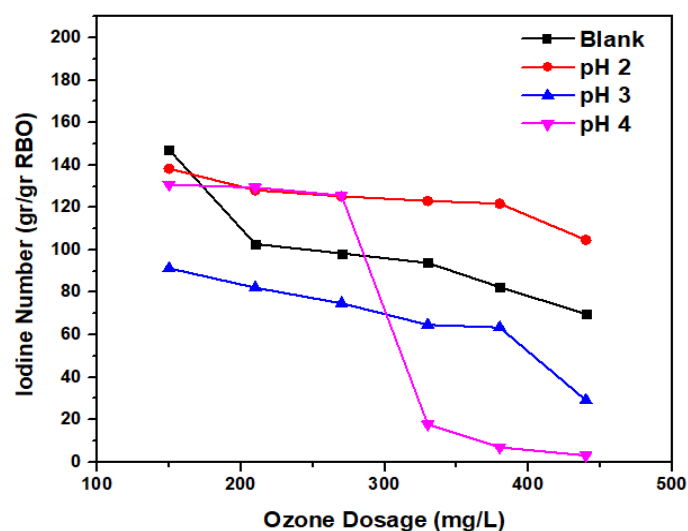


Figure 6. The Effect of pH and Ozone Dosage on Iodine Number of Ozonated RBO

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).

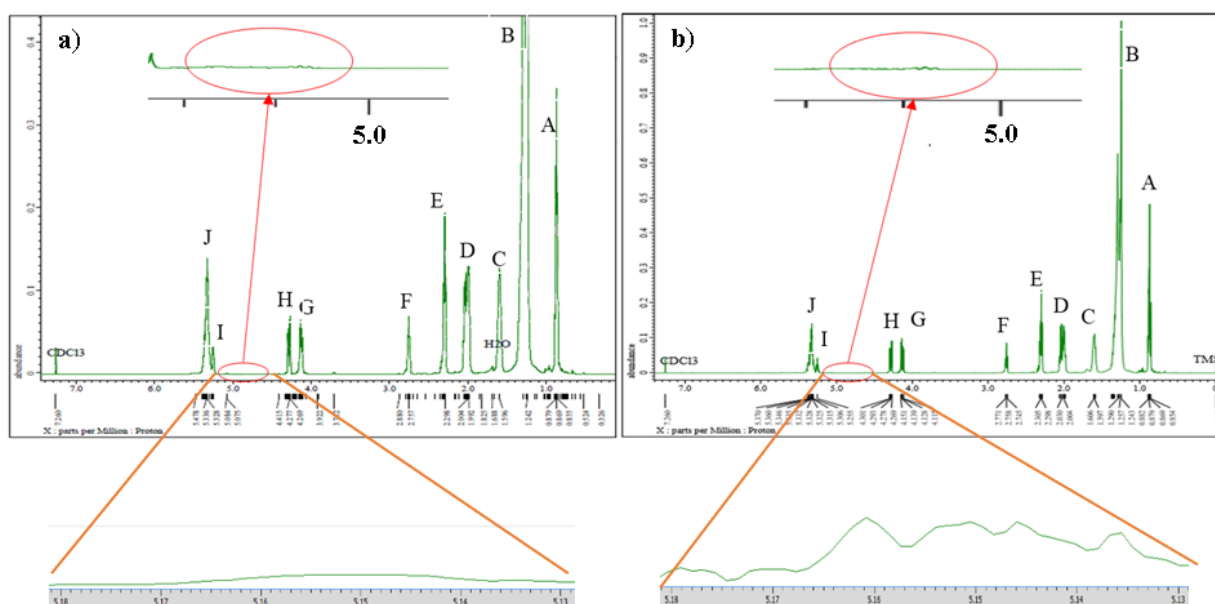
1H and ^{13}C NMR Analysis

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.

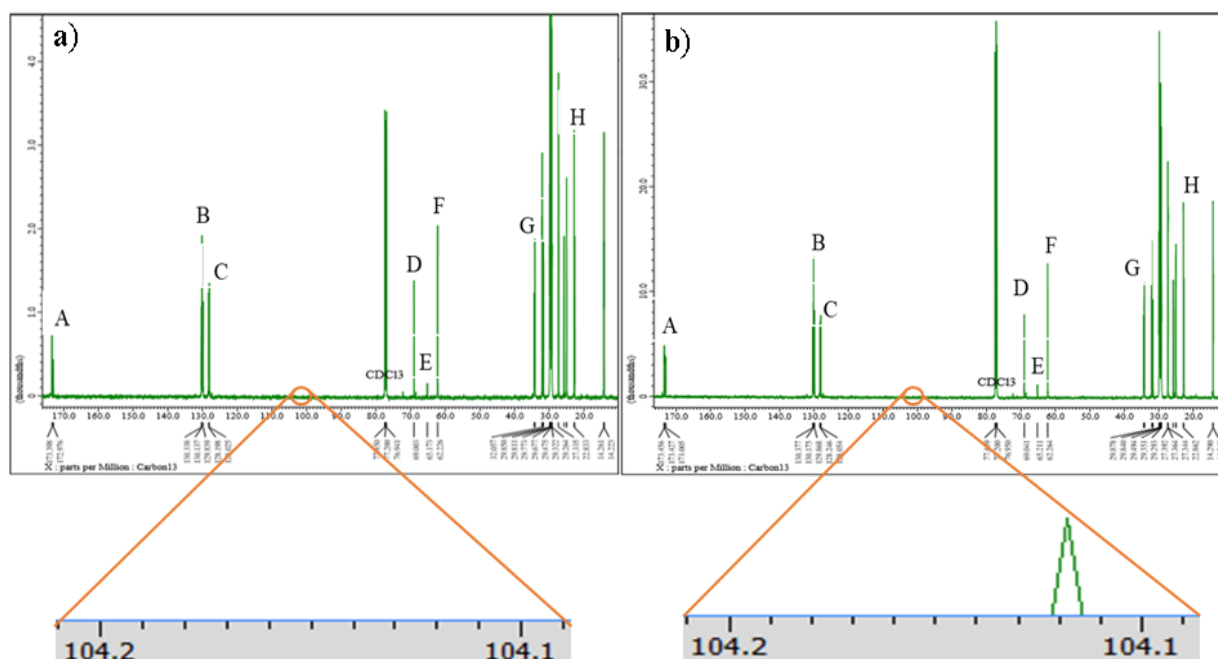


Figure 8. The ^{13}C NMR spectra for RBO a) before and b) after ozonation process.

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.

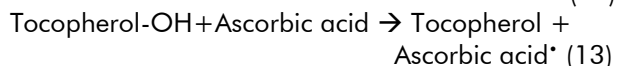
Antioxidant activity and future prospect

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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