Ozonation of Sunflower Oil: Spectroscopic Monitoring of the Degree of Unsaturation

Nestor U. Soriano, Jr.[a], **Veronica P. Migo**[b], **and Masatoshi Matsumura**[a,*]

[a]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, and [b]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Banos, College 4031, Laguna, Philippines

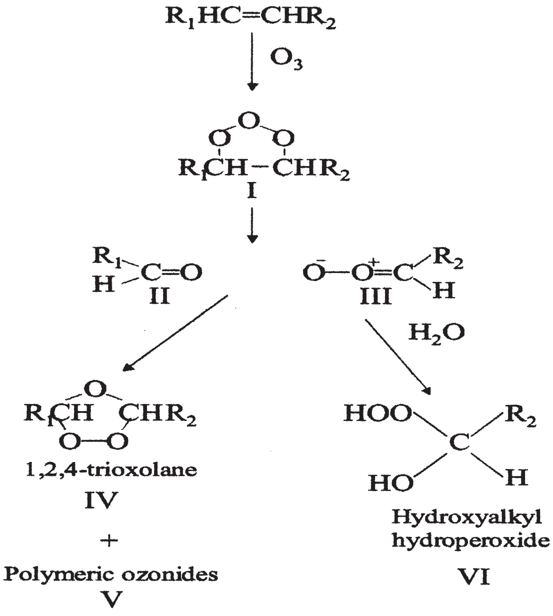
ABSTRACT: Consumption of ozone by unsaturated FA moieties of sunflower oil (SFO) was monitored by ^{1}H NMR and FTIR spectroscopy. Degradation of linoleate was found to be 1.5 and 1.8 times higher than oleate when SFO was ozonized in the absence and presence of water, respectively. Products of ozonation in both cases include aldehydes and ozonides with 1,2,4-trioxolane ring. Hydroxyl-containing compounds, which could be carboxylic acids and/or hydroperoxides, were also detected in samples ozonized in the presence of water. The extent of ozonation had very little effect on the aldehyde/ozonide ratio obtained from NMR spectra, especially in the ozonation of neat SFO. The aldehyde/ozonide ratios obtained regardless of the extent of ozonation were 10.5:89.5 and 46.6:53.4 for SFO ozonized in the absence and presence of water, respectively.

Paper no. J10547 in *JAOCS 80*, 997–1001 (October 2003).

KEY WORDS: Criegee mechanism, FTIR, ^{1}H NMR, ozonation, ozone, ozonides, sunflower oil, triglyceride, 1,2,4-trioxolanes.

The classical Criegee mechanism describing the reaction of ozone with olefins is in general well accepted (Scheme 1). Addition of ozone to the C=C bond produces the unstable initial ozonide (**I**), which decomposes to a carbonyl compound (**II**) and carbonyl oxide (**III**). In the absence of any participating solvent, **II** and **III** recombine to give mainly 1,2,4-trioxolanes and polymeric ozonides. Water molecules may scavenge **III** to give hydroalkyl hydroperoxides (**VI**) if ozonation is carried out in aqueous medium.

Ozonation of vegetable oil followed by reductive decomposition by either hydrogenation over Pd/$CaCO_3$ or Zn/CH_3COOH in ethyl acetate has been reported in early studies to give aldehyde oils (1,2). In more recent studies, FA and their corresponding methyl esters were used as the substrate in ozonation in various media (3–6). Formation of ozonides, carbonyl compounds, hydroperoxides, and other polymeric materials was reported based on the Criegee mechanism. Diaz *et al.* (7) followed the course of consumption of C=C double bonds by ozone in oleic and linoleic acids in two separate systems by ^{1}H NMR spectroscopy. The presence of both these unsaturated FA in TG makes monitoring of ozonation even more complicated. In this paper, the decomposition of linoleate and oleate moieties in neat sunflower oil (SFO) and in the presence of water by ozone was monitored by FTIR and ^{1}H NMR spectroscopy.



SCHEME 1

MATERIALS AND METHODS

Ozonation of SFO. An ozone/oxygen mixture containing about 33–39 g O_3/m^3 was passed through a bubbling reactor containing 50 mL SFO at a rate of 0.50 L/min with stirring. The concentration of ozone bubbled into the reactor was measured every 15 min using a TOA OZ-30 ozone meter (Kobe, Japan). For ozonation with water, 400 mL of deionized water was added with the same amount of SFO, and the ozonation was carried out as above. Formation of a white solid was observed. After ozonation, the oil was bubbled with nitrogen gas for several minutes to remove unreacted ozone. The organic layer was purified by extraction with hexane, centrifugation, drying with anhydrous Na_2SO_4, and evaporation of the extracting solvent. The solid material was washed several times with hexane and dried under the hood.

Spectroscopic analysis. IR spectra were recorded on thin films cast onto KBr plates using a JASCO FTIR 300 spectrometer (Tokyo, Japan). All spectra were the result of 20 scans with a 4 cm^{-1} resolution. For ^{1}H NMR spectroscopy, about 30 mg of the sample was dissolved in $CDCl_3$ containing TMS as standard. Spectra were obtained using a Bruker Avance 600 NMR Spectrometer (Karlsruhe, Germany).

---

*To whom correspondence should be addressed at Institute of Applied Biochemistry, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-0006, Japan. E-mail: aquacel@sakura.cc.tsukuba.ac.jp

RESULTS AND DISCUSSION

Ozonation of neat SFO. Major changes in the functional groups found in SFO and ozonized samples were analyzed by FTIR. Figure 1 shows the IR spectra of SFO and ozonized samples with increasing amounts of consumed ozone. Three characteristic bands of SFO at 725, 1654, and 3008 cm^{-1} due to the presence of C=C bond, diminish with increasing amounts of ozone consumed. This is accompanied by a significant increase in intensity of the band at 1105 cm^{-1}, assigned the C–O stretch of ozonide (6). The strong ozonide band and the absence of an aldehyde band at around 2700–2900 cm^{-1} indicate the extensive formation of 1,2,4-trioxolanes from the combination of carbonyl oxide intermediate and aldehyde formed from the decomposition of initial ozonide, which could be monomeric or polymeric in nature. Although the presence of aldehyde was not observed in IR spectra of ozonized samples, ^{1}H NMR spectroscopy reveals the formation of a small amount of aldehyde especially when water was used as a solvent during ozonation. This may explain the broader band of the carbonyl group at 725 cm^{-1} of the ozonized samples, indicating the presence of new C=O containing compounds. The carbonyl group, moreover, could also be a product of the more complicated oligomerization accompanying ozonation of olefins.

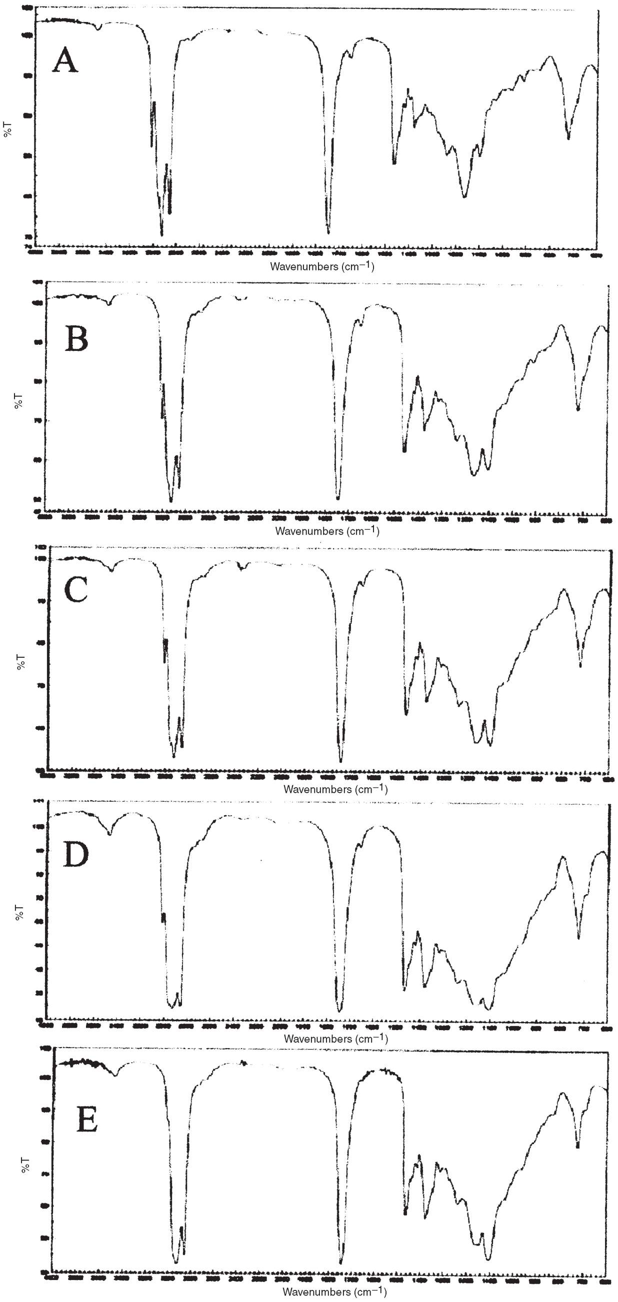


FIG. 1. IR spectra of sunflower oil (SFO) and ozonized SFO (A) SFO; (B) ozonized for 2 h, (C) ozonized for 4 h; (D) ozonized for 6 h; (E) ozonized for 8 h. Ozone absorbed for B, C, D, and E was 0.0437, 0.0832, 0.1223, and 0.1628 g O$_3$/mL oil, respectively).

^{1}H NMR spectroscopy provides more understanding about the degradation of SFO by ozone. Figure 2 shows the ^{1}H NMR spectra of SFO and ozonized SFO. Assignments given for all the signals found in nonozonized oil are as follows: δ (ppm): 0.89, $-\mathrm{C}H_3$; 1.30, $-(\mathrm{C}H_2)\mathrm{n}-$; 1.61, $-\mathrm{C}H_2\mathrm{CH_2OCO}-$; 2.04, $-\mathrm{C}H_2\mathrm{CH{=}CHC}H_2-$; 2.31, $-\mathrm{C}H_2\mathrm{OCO}-$; 2.77, $-\mathrm{CH{=}CHC}H_2-\mathrm{CH{=}CH}-$; 4.15, $-\mathrm{OCOC}H_2\mathrm{CH(OCO)C}H_2\mathrm{OCO}-$; 4.28, $-\mathrm{OCO}-\mathrm{C}H_2\mathrm{CH(OCO)C}H_2\mathrm{OCO}-$; 5.26, $-\mathrm{OCOCH_2C}H\mathrm{(OCO)CH_2}-\mathrm{OCO}-$; 5.35, $-\mathrm{C}H{=}\mathrm{C}H-$. In ozonized samples, the proton in the 1,2,4-trioxolane ring resonates at 5.17 ppm, consistent with the value found in the literature (3–7). The aldehydic proton is also detected at 9.75 ppm.

The degradation of unsaturated FA in SFO by ozone was monitored using the integrated signals in ^{1}H NMR. Integrated signals assigned to the methylene protons in the glyceryl group (4.15 and 4.28 ppm), α-methylene protons adjacent to the carbonyl carbon (2.31 ppm), and the methyl protons (0.89 ppm) were used to calculate the area per proton (8). These four types of protons were considered because their signals are not affected by the FA composition of the TG molecule. The degradation of linoleate ($C_{18:2}$) with increasing amounts of ozone consumed was evaluated by determining the ratio between the signal due to divinyl methylene protons (2.77 ppm) and the area per proton. The degradation of oleate ($C_{18:1}$), on the other hand, was evaluated considering the difference of the signals between olefinic protons (5.35 ppm) and divinyl methylene protons. The production of ozonide and aldehyde was monitored using the signals at 5.17 and 9.75 ppm, respectively.

Figure 3 shows the minimal scission of ozonide to give aldehyde. Degradation of linoleate by ozone was found to be 1.5 times higher than that of oleate. The addition of another unsaturation in linoleate resulted in a relatively small increase in reactivity of the C=C double bond because of the high steric effect on addition of ozone to the double bond. After 8 h of ozonation, resulting in 0.1628 g O$_3$/mL oil, degradation of linoleate was 93% complete as opposed to only 67% for oleate (Fig. 4).

Ozonation of SFO in the presence of water. The IR spectrum of SFO ozonized in the presence of water is shown in Figure 5. Major differences observed in IR spectra of samples

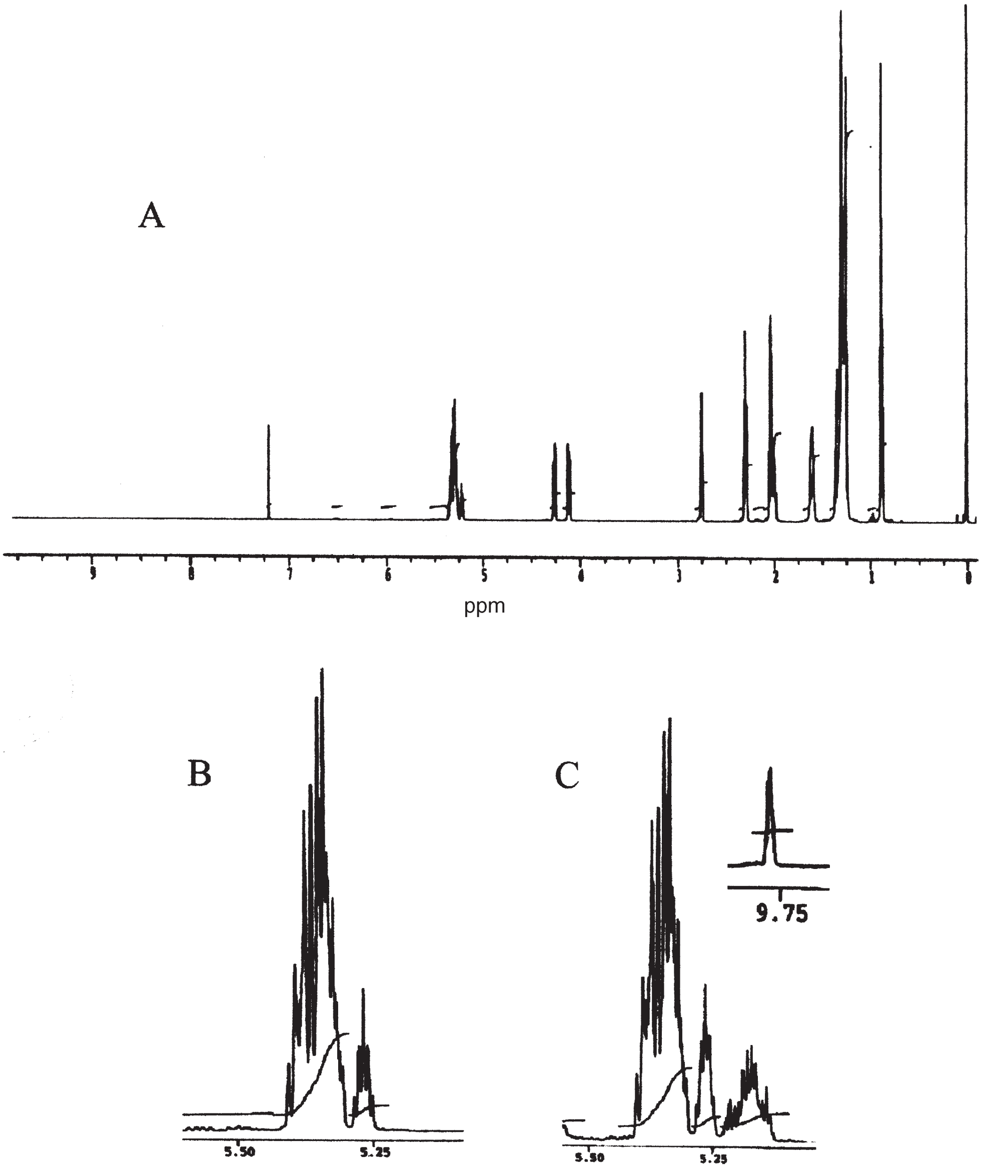


FIG. 2. ^{1}H NMR spectra of SFO and ozonized SFO. (A) SFO; (B) SFO; (C) ozonized SFO, showing signals at 5.17 and 9.75 ppm. See Figure 1 for abbreviation.

ozonized in the presence of water were the appearance of a band at 3471 cm^{-1}, due to the presence of an OH group and the decrease in intensity of the band due to ozonide at 1105 cm^{-1}. Possible types of compounds containing OH groups include carboxylic acids and/or hydroperoxides. Ozonide and aldehyde in ozonized samples were also detected at 5.17 and 9.75 ppm in ^{1}H NMR spectra, respectively. The presence of an α-H of hydroxyalkyl hydroperoxide, which was expected to resonate between 4.9 and 5.1 ppm (5), was not observed.

Degradation of linoleate and oleate was also monitored in the same manner as described above (Fig. 6). Linoleate consumption was 1.8 times higher compared with oleate consumption. In general, the consumption of the same kind of unsaturated FA in SFO by ozone in the absence and presence of water was almost the same. About 53 and 31% of linoleate and oleate, respectively, were consumed after 4 h of ozonation, amounting to 0.0794 g O_3/mL oil (Fig. 7).

Ratio of products. Integrated signals assigned to ozonide and aldehyde at 5.17 and 9.75 ppm in ^{1}H NMR spectra of ozonized samples were used to determine their relative amounts. Table 1 shows the ratio of aldehyde to ozonide at varying extents of ozonation in neat SFO and in the presence of water. In the case of SFO ozonized with water, the ratio obtained was based only on the ^{1}H NMR spectra of the liquid product.

The ratios obtained from ozonation of neat SFO are in closer agreement compared with the ratios obtained when water was

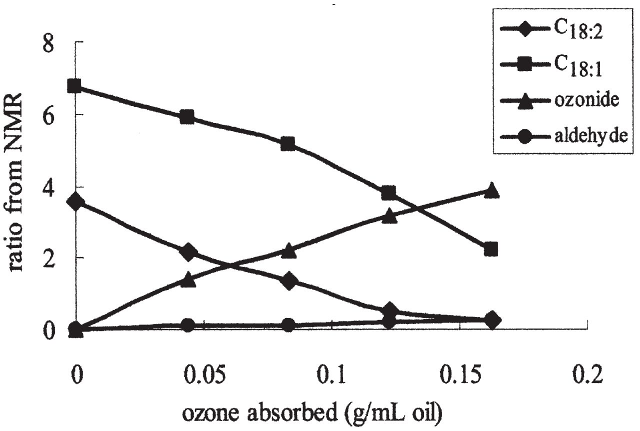


FIG. 3. Consumption of SFO by ozone. See Figure 1 for abbreviation.

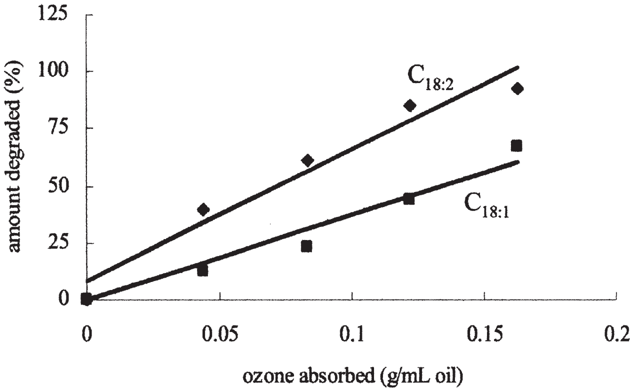


FIG. 4. Degradation of unsaturated FA in SFO by ozone.

added during ozonation. The high reactivity of ozone toward C=C results in uneven degradation of the substrate because of the localization of the reaction around the gas bubbles (9). This effect was expected to be greater in heterogeneous systems, as in the case of SFO and water. In general, however, it appears that the extent of ozonation has little effect on the relative amount of products formed, especially in ozonation of neat SFO. For both cases, the average aldehyde to ozonide ratios obtained regardless of the extent of ozonation were 10.5:89.5 and 46.6:53.4 for SFO ozonized in the absence and presence of water, respectively.

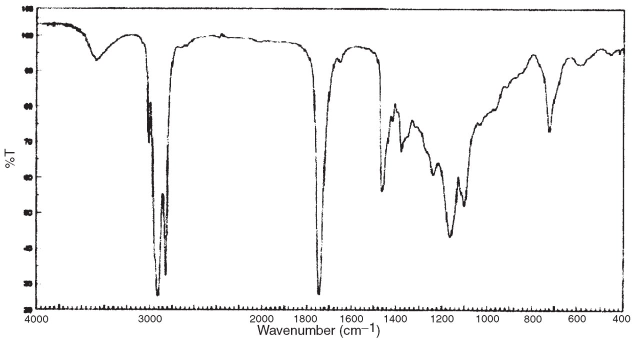


FIG. 5. IR spectrum of SFO ozonized with water (0.0794 g O_3/mL oil). See Figure 1 for abbreviations.

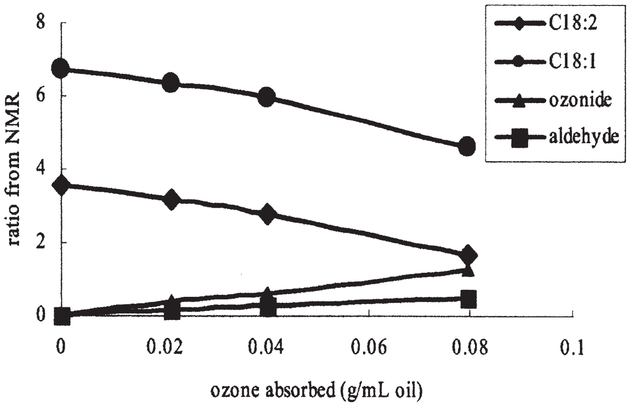


FIG. 6. Consumption of SFO by ozone in the presence of water. See Figure 1 for abbreviation.

The ratios obtained support the results of FTIR analysis that the amount of ozonide was higher when SFO was ozonized neat. According to the Criegee mechanism (Scheme 1), water scavenges the carbonyl oxide intermediate to give hydroxyalkyl hydroperoxide, thereby preventing the formation of ozonide. As already mentioned, however, α-H of this type of hydroperoxide was not detected in ^{1}H NMR analysis. This could be due to the low stability of hydroxyalkyl hydroperoxides, which upon their formation may either lose hydrogen peroxide to give aldehyde or rearrange to carboxylic acid (10). The increase in amount of aldehyde from ^{1}H NMR analysis and the appearance of a band due to an OH group in the IR spectra of

TABLE 1
Relative Amounts of Ozonide and Aldehyde Formed from the Ozonation of Neat SFO and SFO with Water[a]

Neat SFO		SFO + water	
Ozone absorbed (g/mL oil)	Aldehyde/ozonide	Ozone absorbed (g/mL oil)	Aldehyde/ozonide
0.0437	10.5:89.5	0.0214	47.1:52.9
0.0832	10.0:90.0	0.0402	49.3:50.7
0.1223	10.4:89.6	0.0794	43.4:56.6
0.1628	10.9:89.1		

[a] SFO, sunflower oil.

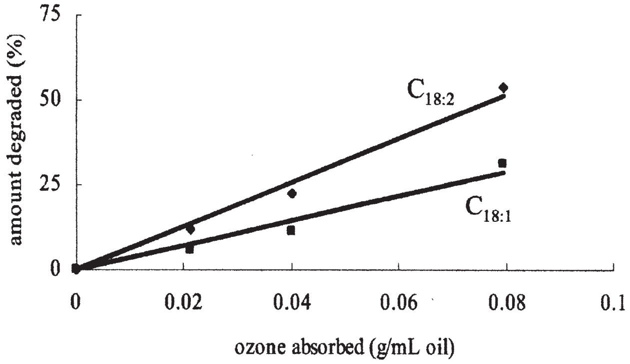


FIG. 7. Degradation of unsaturated FA in SFO by ozone in the presence of water. See Figure 1 for abbreviation.

SFO ozonized with water may support these two fates of hydroxy alkylhydroperoxides.

For ozonation of SFO with water, aside from the viscous liquid product, white polymeric material was also formed whose amount increased with increasing duration of ozonation. For total ozone dosage of 0.0214, 0.0402, and 0.0794 g O_3/mL oil, the amounts of solid material were 1.47, 4.11, and 12.49 g, respectively. Elemental analyses of the solid material collected at various ozonation times showed that it contained about 61.7% C, 8.9% H, and 29.7% O. The theoretical amount of oxygen in the SFO used is only 10.9%. IR analysis also revealed the presence of hydroxyl and carbonyl groups.

The formation of the polymeric material may explain another fate of carbonyl oxide when ozonation was carried out in the presence of water. One possibility is the decomposition of hydroxyalkyl hydroperoxides to their corresponding radicals, hence initiating polymerization of SFO molecules (11). Cunliffe *et al.* (12) demonstrated the emulsion polymerization of olefins after thermolysis at 60°C. They suggested the formation of a hydroxyl hydroperoxide radical as the initiating species of polymerization. This was inferred based on the different final conversion of olefins to polymeric materials, suggesting that an organo peroxide rather than hydrogen peroxide initiates the polymerization.

ACKNOWLEDGMENT

This research was conducted with support from a New Energy and Industrial Technology Development International Joint Research Grant.

REFERENCES

1. Pryde, E.H., D.E. Anders, H.M. Tuter, and J.C. Cowan, Unsaturated Aldehyde Oils by the Partial Ozonation of Soybean Oil, *J. Am. Oil Chem. Soc. 40*:497–499 (1963).
2. Sharpe, R.E., and D.A. Berry, Instrumental Studies of Aldehyde Oils and Their Reaction with Selected Amines, *Ibid. 42*:835–838 (1965).
3. Ledea, O., J. Molerio, M. Diaz, D. Jardines, A. Rosado, and T. Correa, Analysis of Ozonides and Peroxidic Compounds from Methyl Oleate Ozonation, *Revista CENIC Ciencias Quimicas 29*:75–78 (1998).
4. Nishikawa, N., K. Yamada, S. Matsutani, M. Higo, H. Kigawa, and T. Inagaki, Structures of Ozonolysis Products of Methyl Oleate Obtained in a Carboxylic Acid Medium, *J. Am. Oil Chem. Soc. 72*:735–740 (1995).
5. Rebrovic, L., The Peroxidic Species Generated by Ozonolysis of Oleic Acid or Methyl Oleate in a Carboxylic Acid Medium, *Ibid. 69*:159–165 (1992).
6. Wu, M., D.F. Church, T.J. Mahier, S.A. Barker, and W.A. Pryor, Separation and Spectral Data of the Six Isomeric Ozonides from Methyl Oleate, *Lipids. 27*:129–135 (1992).
7. Diaz, M., F. Hernandez, I. Alvarez, H. Velez, O. Ledea, and J. Molerio, The ^{1}H NMR Spectroscopy in Following Ozone Reaction with Unsaturated Fatty Acids, *Revista CENIC Ciencias Quimicas. 29*:89–93 (1998).
8. Miyake, Y., K. Yokomizo, and N. Matsuzaki, Determination of Unsaturated Fatty Acid Composition by High-Resolution Nuclear Magnetic Resonance Spectroscopy, *J. Am. Oil Chem. Soc. 75*:1091–1094 (1998).
9. Anachkov, M.P., S.K. Rakovski, and R.V. Stefanova, Ozonolysis of 1,4-*cis*-Polyisoprene and 1,4-*trans*-Polyisoprene in Solution, *Polym. Deg. Stab. 67*:355–363 (2000).
10. Bailey P.S., *Ozonation in Organic Chemistry*, Academic Press, New York, 1978, Vol. 1, pp. 111–113.
11. Nor, H.M., and J.R. Ebdon, Ozonolysis of Natural Rubber in Chloroform Solution. Part I. A Study by GPC and FTIR Spectroscopy, *Polymer. 41*:2359–2365 (2000).
12. Cunliffe, D., J.E. Lockley, J.R. Ebdon, S. Rimmer, P. Palasz, and S. Emmett, Emulsion Polymerization of Methyl Metacrylate Initiated by Alkene Ozonates, *Ibid. 42*:9329–9333 (2001).

[Received January 21, 2003; accepted July 31, 2003]