

## Spectroscopic Evidence of the Autoxidation Products Derived from Methyl Linolenate

Jong-Kyoon Ahn, Mi-Za Cho,\* In-Sook Kim\*\* and Sung-Ki Oh\*

\*International College of Hotel Administration, Kyung Hee University, Seoul, Korea

\*\*Department of Food and Nutrition, Won Kwang University, Iri, Korea

\*Institute of Food Development, Kyung Hee University, Suwon, Korea

## Methyl Linolenate의 酸化生成物에 對한 分光學的 研究

安鍾均 · 趙米子\* · 金仁淑\*\* · 吳成基

\*慶熙大學校 호텔經營專門大學, \*\* 圓光大學校 食品營養學科, 慶熙大學校 食糧資源開發研究所

### 초 록

Methyl linolenate를 自動酸化反應의 model 化合物로 選定하여 室溫에서 90ml/min.의 air flow rate로 96時間 酸化시켰으며 여기서 얻어진 酸化生成物을 TLC로 分雜한 다음 各 分劃物을 ir, uv, nmr로 分析하였다. 分光機器分析 結果는 서로 一致하였으며 Rf. 0.28과 0.15~0.10의 化合物들이 hydroperoxide와 cis, trans-substituted diene인 것으로 確認되었고 主로 cis, trans-conjugated diene system과 若干의 trans, trans-configuration인 것으로 밝혀졌다. 特히 5.83~6.60ppm에서 나타난 multiplet 部位는 酸化生成物들의 化學構造를 決定함에 있어서 큰 도움을 줄 것으로 생각하기 때문에 앞으로 많은 研究가 要望된다.

### Introduction

It has been well known that triunsaturated fatty acids are present in a larg enumber of vegetable oils and are of biological importance in the body.<sup>1,2)</sup> Considering the fact that the reversion flavors of soybean oil are mainly caused by linolenic acid,<sup>3-5)</sup> it seems of great interest to investigate the autoxidation products of this acid. Not only the oxidation of linolenic acid leads to the formation of conjugated dien hydroperoxide, but also yields bicyclic endoperoxide which forms in turn prostaglandins.<sup>6-11)</sup> The mechanism of the autoxidation of methyl linoleate and linolenate was reported by several

workers.<sup>12-14)</sup> It is generally accepted that these autoxidations rae free-radical chain reaction.

Recently, prostaglandin has been paid great attention to research workers, because of its regulatory effect on hormone, nerve and stimulation, as well as its pharmaceutical effect such as labor at the end of pregnancy, ulcer, high blood pressure, rheumatoid arthritis, asthma, nasal congestion and certain viral diseases.

A variety of analytical methods have been used for the studies on the autoxidation of linoleic and linoleic acids.<sup>6,7,9,12-24)</sup> Nevertheless, NMR spectrometry has been utilized in a very few cases as an analytical tool for the autoxidation of unsaturated fatty acids.<sup>7,25)</sup> The objective of this paper was to isolate and characterize the oxidation products derived from methyl linolenate and also to examine the propriety of the application of NMR spectrometry to the analysis of the oxidation products.

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Corresponding author: S.K. Oh

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### Materials and Methods

#### Materials

All the chemical reagents used were special grade and purchased from Merck Company, if not specified. The materials used were methyl linolenate (99% pure, Tokyo Kasei Co. iodine value of 237), analytical TLC plate F 254, 0.02×10×10cm), silica gel HF 254, n-hexane, ethyl ether, tetramethylsilane, chloroform, and deuteriochloroform.

#### Methods

*Determination of iodine value(I.V.).* The iodine value of authentic methyl linolenate was determined by the method of AOACWijs.<sup>26)</sup>

*Oxidation of methyl linolenate.* Methyl linolenate(1.5g) was in a test tube attached to a capillary tube and aerated at the flow rate of 90ml/min by using air compressor. The aeration was continued 96 hrs at room temperature.

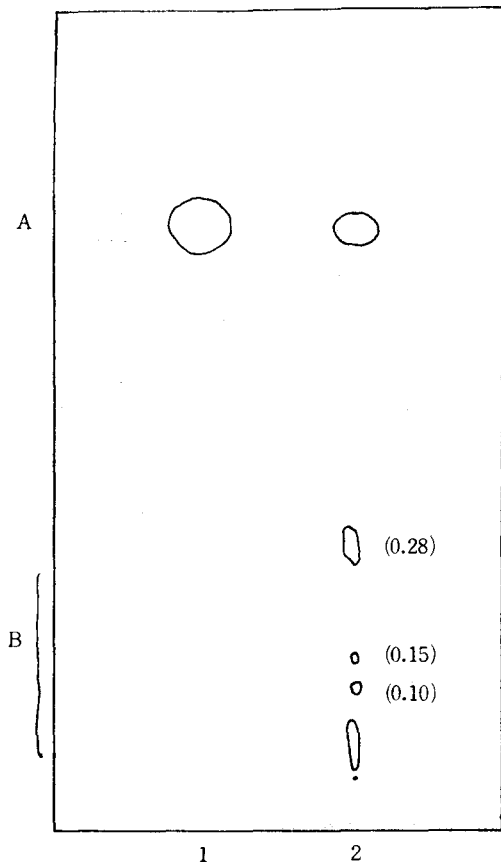
*Thin-layer chromatography.* Silica gel was coated on a glass plate(0.1×20×20cm) by using an applicator and dried 18hrs at room temperature, followed by activation at 110°C for one hour. A mixed solvent system of n-hexane and diethyl ether(80 : 25) was used,<sup>19)</sup> and the spots isolated were detected by using UV lamp(short wave).

UV absorption measurements of initial and autoxidized sample in n-hexane(99% pure) were made in the range from 220 to 350nm, by using UV spectrophotometer(Hitachi, Model 200-20). The IR absorptions of initial and autoxidized samples were measured in the range from 500 to 4000cm<sup>-1</sup>, by using IR spectrophotometer (Nicolet, FTIR M<sub>X</sub>-1, Model 80), and KBr salt plate(neat liquid) was used.

Each of initial and autoxidized samples was placed in NMR tube, and chloroform containing 5% TMS(v/v) was added and measured the chemical shifts by using NMR spectrometer (Varian T-60, 60MHz).

### Results and Discussion

As shown in Fig. 1, methyl linolenate oxidized for 96 hrs showed at least 5 spots on an analytical TLC plate. The spot of Rf 0.76 was identified as unreacted methyl linolenate, and the others(Rf 0.03, 0.10, 0.15 and 0.28) were thought to be the most likely the autoxidation products in question. The sample was also chromatographed on a preparative TLC glass plate. All the spots, except only 2 spots (Rf 0.10 and 0.15) appeared on an analytical TLC plate, were completely separated, and they



1. Authentic methyl linolenate
2. Methyl linolenate aerated for 96hrs

- A. Methyl linolenate
- B. Oxidation products

Developing solvent: n-hexane: ethyl ether=80 : 25

Fig. 1. Thin-layer chromatography of oxidized methyl linolenate

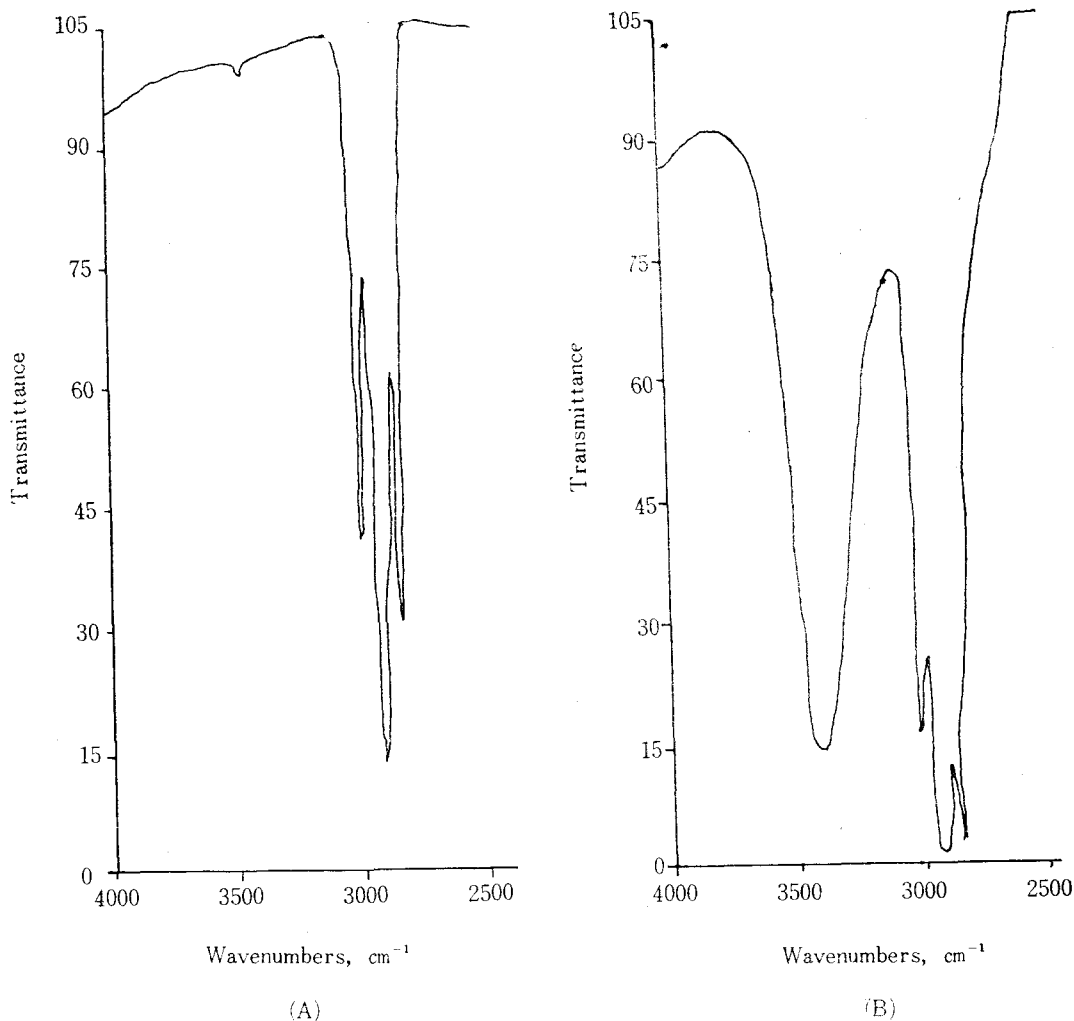


Fig. 2. Infrared spectra of methyl linolenate(A) and oxidation product(B) isolated by TLC(Rf. 0.28)

were fractionated as usual. The fraction of Rf 0.28 was then characterized by IR spectrophotometer. The spectrum was different from that of unoxidized authentic sample by showing 3 new absorption bands at 3422, 985 and 950 $\text{cm}^{-1}$  (Fin. 2 and 3). The absorption band at 3422  $\text{cm}^{-1}$  corresponds to the -OH stretching vibration, and the bands at 950 and 985 $\text{cm}^{-1}$  indicate the presence of a trans, cis-substituted conjugated diene.<sup>14,21)</sup> As shown in Fig. 4, the fraction of Rf 0.28 showed the absorption maximum at

232nm, indicating the presence of diene compound. The absorption maximum at 265nm corresponding to triene compound, however, was not observed in the UV spectra.<sup>13)</sup> It seems likely that the results are strongly supported by NMR spectrum which evidences the presence of hydroperoxide by showing a newly appeared peak at 4.10ppm, as well as the presence of conjugated double bond system and/or the decomposition products such as prostaglandinlike endoperoxides via cyclization at 5.38~6.60ppm (Fig.

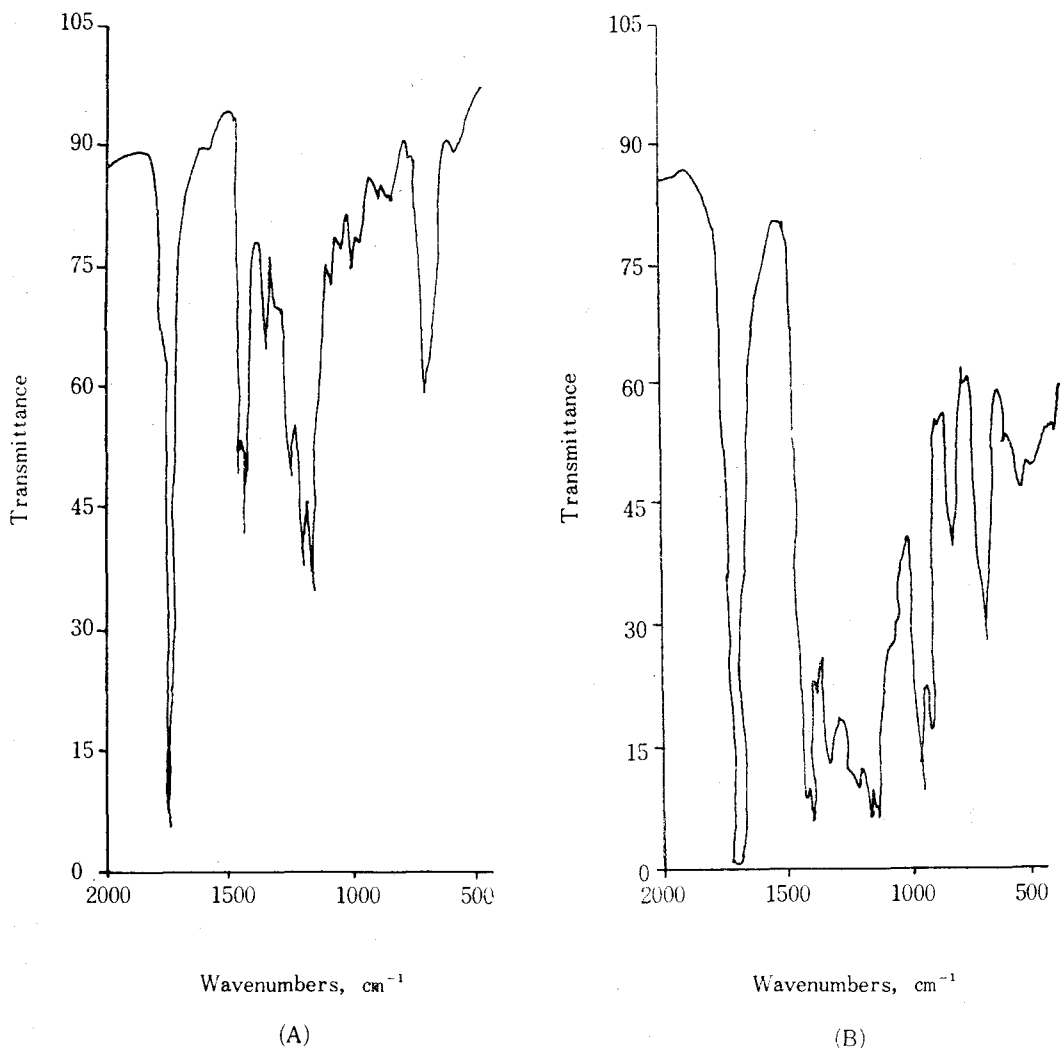


Fig. 3. Infrared spectra of methyl linolenate(A) and oxidation product(B) isolated by TLC(Rf 0.28)

5). It should be noted, however, that the multiplet splitting pattern in the range of 5.38~6.60ppm still remains to be explored.

The IR, UV and NMR spectra of the fractions of Rf 0.10 and 0.15 were very similar to those of the fraction of Rf 0.28. In contrast to the IR absorption spectrum of the fraction of Rf 0.28, however, the fractions of Rf 0.10 and 0.15 showed the absorption bands at 987 and 1045cm<sup>-1</sup>(Fig. 6). The band at 987cm<sup>-1</sup> is characteristic of a trans, trans-conjugated diene, and the band at 1045cm<sup>-1</sup> indicates the presence

of -OOH group. The results suggest that the autoxidation of methyl linolenate produces cis, trans-and trans, trans-conjugated dienehydroperoxides. Thus, infrared analyses show that the autoxidation products of methyl linolenate have a predominant cis, trans-conjugated diene system with some trans, trans-configuration which are also evidenced by TLC chromatograms. In conclusion, the spectroscopic data of IR, UV and NMR are consistent with each other, and further investigation for the multiplet region of 5.83~6.60ppm would be very helpful in the character-

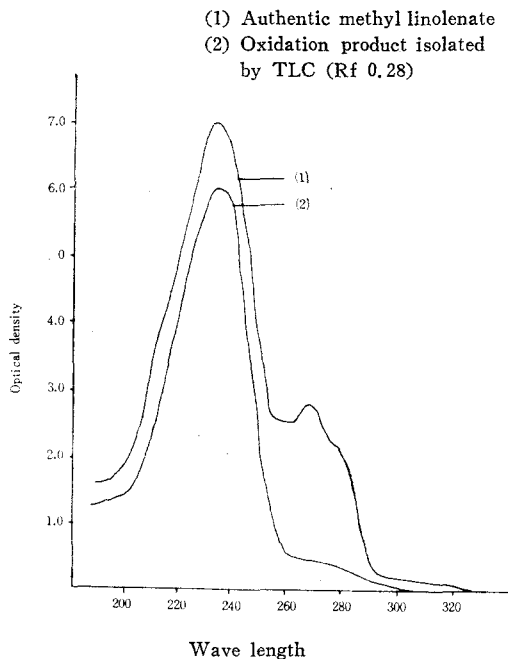


Fig. 4. UV spectra of the oxidation product of methyl linolenate

rization of the oxidation products derived from triunsaturated fatty acids.

### Abstract

The autoxidation products derived from methyl linolenate are isolated and characterized by using thin-layer chromatography, infrared, ultraviolet and nuclear magnetic resonance spectrometries. In addition, the propriety of the application of NMR spectrometry to the analysis of the autoxidation products is examined. Spectroscopic data indicate that the autoxidation of methyl linolenate produces hydroperoxides and trans, cis-substituted diene. The autoxidation products have a predominant cis, trans-conjugated diene system with some trans, trans-configuration. The spectroscopic data of ir, uv, and nmr are very consistent with each other, and further investigation of the multiplet region at 5.83~6.60ppm would be very helpful for the structural elucidation of

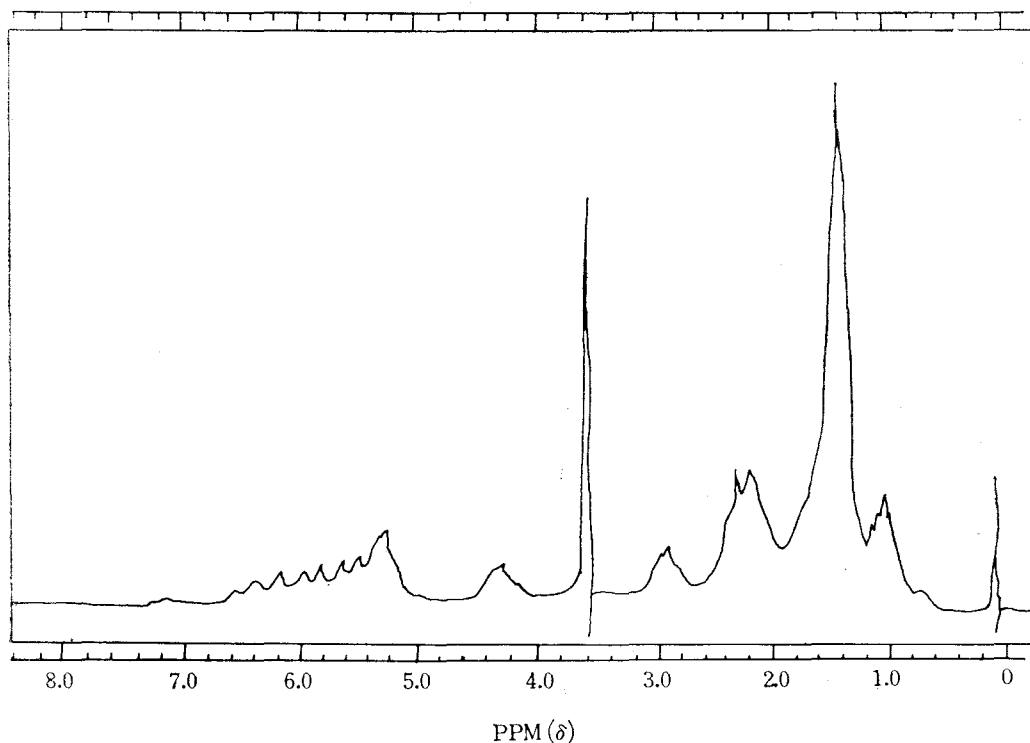


Fig. 5. NMR spectra of the oxidation product of methyl linolenate

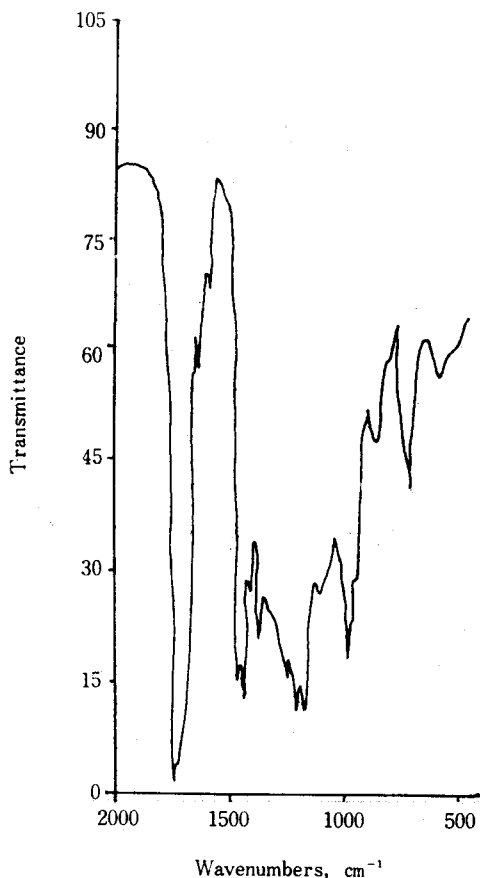


Fig. 6. Infrared spectra of the oxidation product of methyl linolenate isolated by TLC(Rf 0.15~0.10)

the autoxidation products.

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