# Determination of Degree of Oxidation of Methyl Linoleate and Linolenate by Weighing Method

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**ABSTRACT:** Autoxidation of methyl linolenate and linoleate was investigated at room temperature in the light and dark during a period of 2 mon using a weighing method accompanied with $^1$H NMR measurements. In the light, the increase in weight, or autoxidation process, of the two compounds began immediately after the start of the experiment. In the dark, the weight of methyl linolenate began to increase after a delay of 1 wk, and the weight of methyl linoleate after 40 d. The maximal increases in the weights of the compounds were somewhat greater in the dark than in the light; the weight of methyl linolenate in the dark increased by 22.3% and in the light by 20.8%. The corresponding values for methyl linoleate were 16.9 and 14.4%, respectively. Results of the NMR measurements were compared to the weighing results, and a table was constructed representing the correlation between selected multiplets and corresponding weight increments.

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**KEY WORDS:** Linoleate, linolenate, weighing method and oxidation.

Several methods are available for determining the degree of oxidation of PUFA. Among them are the active oxygen method, the Schaal oven test, the Rancimat method, and the determination of peroxide, anisidine, carbonyl, and acid values (1). However, the simplest method, namely, the one in which progress of oxidation is detected by weighing the sample periodically, is a rarely used technique (2–4). A probable reason for this is that the method itself does not provide information about the underlying chemical processes. In any case, it is an illustrative and quite practical method. In the beginning of a reaction, when there are no volatile decomposition products present, the method gives an exact value for the degree of oxidation (defined as the percent weight gain from the starting mass). The method has been successfully used in the determination of the antioxidative activity of α- and γ-tocotrienols in methyl linoleate (4).

In the present study, the weighing method was used to examine the progressive autoxidation of methyl linoleate and linolenate. The esters were used instead of free acids because they are easier to purify. The measurements were accompanied by NMR measurements.

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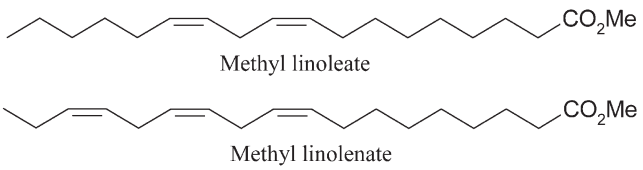
## EXPERIMENTAL PROCEDURES

The degree of oxidation of methyl linoleate and methyl linolenate (Scheme 1) was monitored at room temperature both in the light and in the dark during a period of about 2 mon by weighing the samples daily. The measurements were carried out during the dark season between November 25, and December 29, 2000, in Helsinki, Finland. Methyl linolenate was prepared from linseed oil by means of transesterification with methanol. The ester was purified using argentation chromatography followed by normal flash silica chromatography. The purity was checked with the aid of TLC and $^1$H NMR. Methyl linoleate was prepared analogously starting from sunflower seed oil.




**SCHEME 1**

Immediately after preparation, samples of esters were weighed into open Petri dishes and the oxidation tests were started. An analytical balance was used for weighing. The weights of the samples were: methyl linolenate sample 1 = 410.9 mg and sample 2 = 382.4 mg, methyl linoleate sample 3 = 406.6 mg and sample 4 = 405.1 mg. The precision of the balance was 0.1 mg.

Samples 1 and 3 were exposed to an ordinary fluorescent lamp (L58W/31 LUMILUX; Osram GmbH, Munich, Germany) at a distance of about 1 m. The lamp was turned on during normal working days from 9 A.M. to 5 P.M. The illumination on the sample was 760 lx. Outside these periods, the illumination was scant because of the dark season in Finland. Samples 2 and 4 were kept in the dark and shielded against exposure to light during weighing with perforated foils. All the samples were stored at a temperature of 20 ± 2°C.

The oxidation process of methyl linolenate was also investigated by $^1$H NMR spectroscopy to identify the connection between the degree of oxidation of the sample as determined by the weighing method and the corresponding structural changes of the molecule. For this purpose, samples with weight gains of 0, 1, 5, 10 and 15% were studied by $^1$H NMR measurements.

## RESULTS AND DISCUSSION

Changes in the weight of the samples as a function of time are shown in Figure 1. In the case of illuminated methyl linolenate (sample 1) the weight began to increase immediately after the beginning of the experiment, whereas for the same compound kept in the dark (sample 2), a delay of 7–8 d was observed. In the case of illuminated methyl linoleate (sample 3), the delay was about 1 d. The delay for the same compound (sample 4) kept in the dark was about 40 d.

The maximal increase in the weight of the samples kept in the dark was somewhat greater than the increase in the samples kept in the light (22.3% for methyl linolenate in the dark and 20.8% in the light; 16.9% for methyl linoleate in the dark and 14.4% in the light). The probable explanation for the lesser weight increase of the light-exposed samples is the light-catalyzed dissociation of formed hydroperoxides. Our results suggest that, on the average, four oxygen atoms are joined to one methyl linolenate molecule and three to one methyl linoleate molecule. Further, the slopes of the curves in the above figure reveal that the autoxidation speed of the linolenates is somewhat greater and the speed of the degradation of the product somewhat smaller than the corresponding speeds of the linoleates.

The autoxidation (1) of FA is considered to begin by abstraction of a hydrogen radical from the internal allylic position between the two double bonds (Fig. 2). The dissociation energy of the C–H bond of this bis-allylic internal methylene is lower than that of the external allylic methylenes. For this reason, hydrogen radical abstraction favors the internal allylic site during the initiation step of the reaction. The unpaired electron of the newly formed radical delocalizes in the double-bond system, and thereafter, an atmospheric oxygen molecule is connected as shown in Figure 2.

Methyl linolenate has four reactive allylic positions per molecule, whereas methyl linoleate has three. Thus, the more rapid oxidation of the former is understandable. On the other hand, the degradation and volatilization of methyl linolenate is slower because it may undergo intermolecular addition reactions or cross-linking reactions more easily and thus the formation of volatile compounds is reduced.

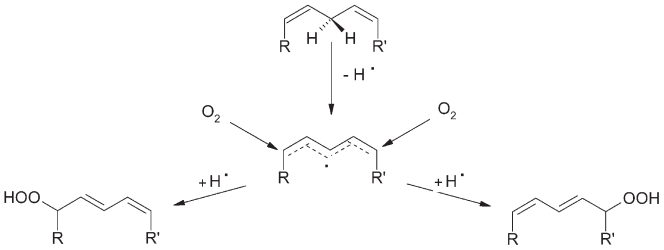



**FIG. 2.** Probable mechanism of the initial step of autoxidation of unsaturated FA (1).

The $^1$H NMR spectra of linolenate samples with 0 and 10% weight increase are shown in Figures 3 and 4. The double bond system absorption regions where double-bond systems are evident in the spectra are magnified to illustrate the oxidation process after the initiation step. Table 1 presents the integrals of the most important multiplets in the spectra of linolenate samples, starting from the initiation of the oxidation reaction, and the corresponding mass increases.

Examination of the spectra in Figures 3 and 4 and of Table 1 reveals that about one-half of the allylic methylenes have reacted when the mass has increased by 10%. This is clearly seen by comparing the integrals of the nonreacting ester methoxy methyl ($\delta$ = 3.7 ppm) signal with integrals of the bis-allylic ($\delta$ = 2.7–2.9 ppm) and allylic $\delta$ = 2.0–2.2 ppm) methylene signals. Further, the bis-allylic, i.e., internal, methylenes seem to be favored over the external methylenes as expected, but only to a moderate extent. The ratio of the integrals of bis/mono is only *ca.* 4:3.

Most of the NMR signals originating from the reaction products can be explained as hydroperoxy structures: $\delta$ 8.8–8.2 ppm, hydroperoxy protons; $\delta$ 6.8–5.8 ppm, olefinic

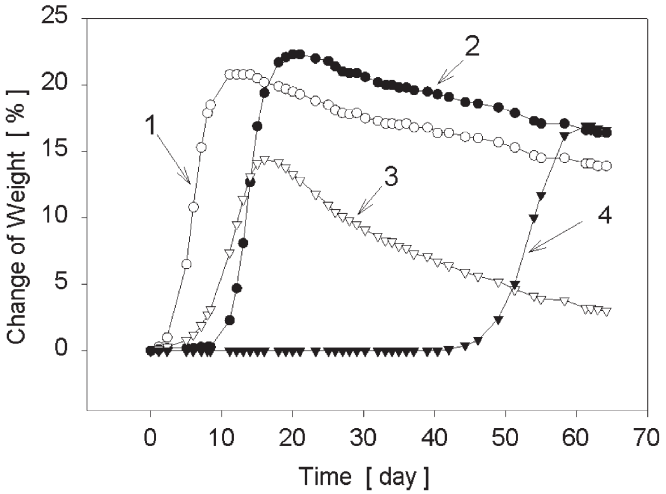



**FIG. 1.** Changes in the weight of methyl linolenate in the light (1) and in the dark (2) and changes in the weight of methyl linoleate in the light (3) and in the dark (4).

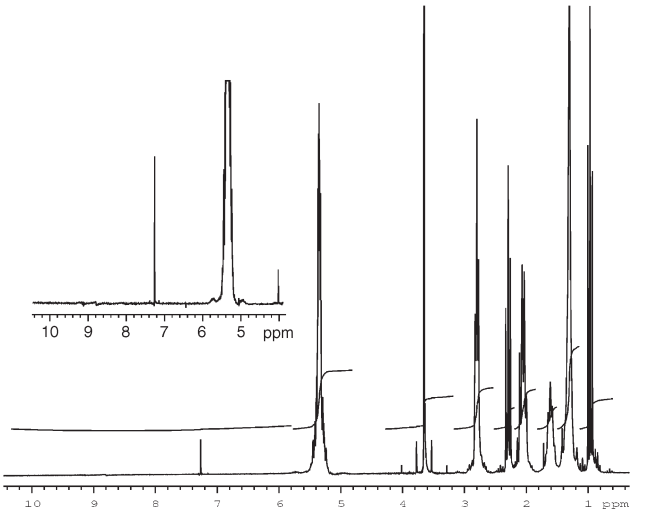



**FIG. 3.** The $^1$H NMR spectrum of methyl linolenate before oxidation (200 MHz, Varian Gemini 2000, $CDCl_3$, *ca.* 30 mg/mL, 20°C).

**TABLE 1**
**Integrals of Selected $^1$H NMR Spectrum Multiplets, and the Corresponding Oxidation Degrees of Methyl Linolenate Represented as Weight Increments[a]**

| Increase of mass (%) | Multiplet at 2.0–2.2 ppm[b] | Multiplet at 2.2–2.4 ppm[c] | Multiplet at 2.7–2.9 ppm[d] | Multiplet at 3.7 ppm[e] | Multiplet at 5.2–5.6 ppm[f] |
|---|---|---|---|---|---|
| 0.0 | 4 | 2 | 4 | 3 | 6 |
| 1.0 | 4 | 2 | 4 | 3 | 5⅔ |
| 5.0 | 3⅔ | 2⅔ | 3⅓ | 3 | 5 |
| 10.0 | 3 | 2⅔ | 2⅔ | 3 | 4 |
| 15.0 | 2 | 2⅔ | 1¾ | 3 | 2 |

[a]A proton is used as integration unit and methyl ester as reference.
[b]External allylic methylene.
[c]α-Methylene.
[d]Internal allylic methylene.
[e]Methyl ester.
[f]Methylene-interrupted double bond.

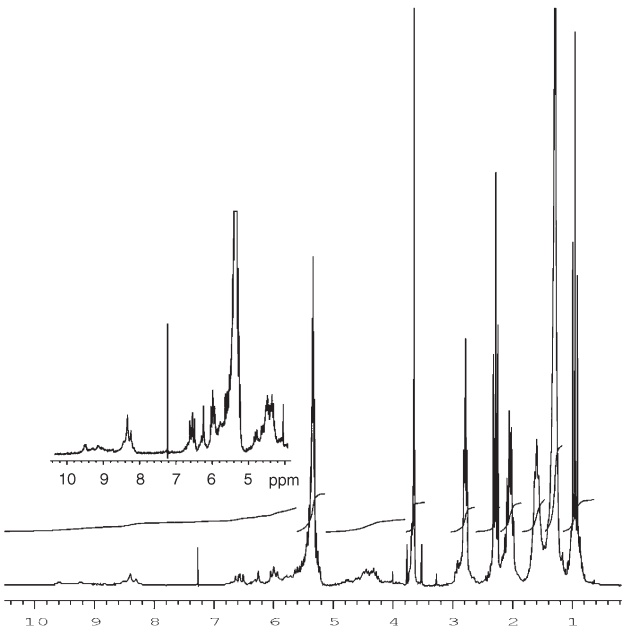



**FIG. 4.** The $^1$H NMR spectrum of 10% weight gain methyl linolenate in the light (200 MHz, Varian Gemini 2000, $CDCl_3$, *ca.* 30 mg/mL, 20°C).

protons of conjugated *E,Z*-dienes; and δ 4.8–4.2 ppm, protons α to hydroperoxide methines (5).

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