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Isomeric Composition of Hydroperoxides Formed by Autoxidation of Adlay Lipid and Triglyceride

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Abstract

The compositions of hydroperoxy fatty acid components of lipid in raw adlay powder(RAP) and processed adlay powder(PAP) stored at 35℃ and RAP lipid and triglyceride(TG) autoxidized 35℃ were studied. During autoxidation, the time taken to reach 100 of peroxide value was estimated as 175 days in RAP lipid and 80 days in TG. The 9-hydroperoxide and 13-hydroperoxide isomers were the major hydroperoxy fatty acids found in oxidized adlay lipid. Lower levels of 8-,10-,11-,12- and 16-hydroperoxide isomer were also observed. The compositions of hydroperoxy fatty acid components obtained from autoxidized RAP lipid and TG were similar to those of hydroperoxy fatty acid components in lipid extracted from stored RAP and PAP.

Introduction

Adlay(Coix Lachryma-jobi Linne var. Mayuen (Roman) Stapf, 율무) belonged to *Graminaceae* was used as food and medicine and its powder is recently used as adlay tea. The lipid content of adlay represents a small fraction, but this lipid undoubtedly plays an important functional role in their products during storage[1].

It is well known that autocatalytic autoxidation of lipid proceeds by free radical chain reaction resulting in the accumulation of hydroperoxides and a variety of secondary oxidation products. Earlier works have demonstrated that isomeric compounds of hydroperoxides are formed by autoxidation of unsaturated fatty acids[2]. The isomeric compositions of hydroperoxides formed by autoxidation of methyl oleate, methyl linoleate and methyl linolenate have already been investigated in detail by high performance liquid chromatography (HPLC)[3-4] and gas chromatography-mass spectrometry(GC-MS)[5-7].

However, only a few investigations have been carried out to characterize the isomeric compositions of hydroperoxides of oxidized vegetable oil[8-9]. This study was performed to clarify the structure of isomeric compositions of hydroperoxy fatty acid components formed by oxidation of adlay lipids.

Materials and Methods

Materials and autoxidation

The preparation of raw adlay powder(RAP) and processed adlay powder(PAP) and RAP lipid and TG were same as described in previous paper[10]. RAP and PAP were stored at 35℃ for four months, and RAP lipid and TG in a glass vial were autoxidized by incubating at 35℃ in the dark. The peroxide value was measured according to the method of Asakawa and Matsushida[11].

Isolation of hydroperoxides

Oxidized lipid and TG were applied to a TLC

plate(1.25mm thick) coated with silica gel(silica gel PF-254, Merck Co., Germany) and developed with the solvent of hexane-ethyl ether-acetic acid (8 : 7 : 0.1, v/v/v). The bands of hydroperoxides were detected under ultraviolet light and were scrapped off and then extracted with chloroform three times. IR spectrum and H-NMR spectrum were taken with a Perkin-Elmer 1330 spectrophotometer and a Varian EM-360(60MHz) spectrophotometer, respectively.

Determination of the isomeric compositions of hydroperoxy fatty acid components

Hydroperoxides isolated from TG and lipids were reduced by sodium borohydride in methanol. Cataytic hydrogenations of 0.5g samples were carred out in 95% ethanol with PtO_2 at atmospheric pressure. Hydrogen uptake was followed manometrically and continued 10 to 15 min after absorption ceased. Hydroxy ester derivatives were methylated with 28% sodium methoxide in benzene.

The isomeric compositions of the hydroperoxy fatty acid components were determined by GC and GC-MS. GC-MS analysis was carried out with a JEOL DX-300(Japan) equipped with a fused silica OV-101 capillary column(25m×0.2mm, i.d.) and programmed from 180℃ to 300℃ at 3℃/min. Relative peak areas were determined by GC with a flame ionization detector under the same conditions and were reported as a percentage.

Results and Discussion

Changes of peroxide value

Fig.1 shows the changes of peroxide value in RAP lipid and TG during autoxidation. Prior to the autoxidation process, peroxide values of RAP lipid and TG were lower than 1.0meq/kg. RAP lipid appeared to be highly resistant to oxidation as compared with TG. The time taken to reach 100 of peroxide value was estimated as 175 days in RAP lipid and 80 days in TG. It is apparent that TG is more susceptible to oxidation than RAP lipid because of the lack of tocopherol in TG. It seemed likely that the stability of lipids to oxidation is affected by their tocopherol contents, peroxide contents and fatty acid compositions, especially linoleic acid and linolenic acid.

Fatty acids of RAP lipid were determined as palmitic acid(15.4%), stearic acid(1.8%), oleic acid(42.3%), linoleic acid(38.8%) and linolenic acid(1.1%), and those of soyben a oil were shown as palmitic acid(9%), stearic acid((4%), oleic acid (23%), linoleic acid(56%) and linolenic acid(8%) [9]. The results indicating that RAP lipid was more stable to oxidation than soybean oil can be explained by the difference of fatty acid compositions, especially linoleic acid and linolenic acid.

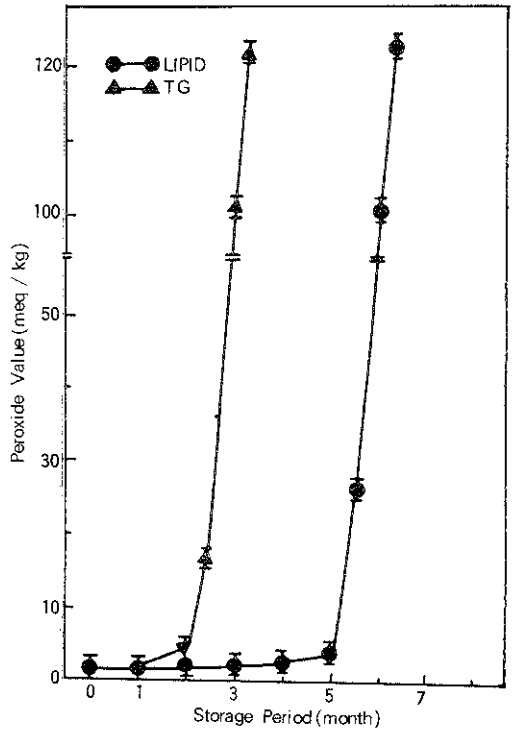

Fig. 1. Changes of peroxide value of raw adlay powder(RAP) lipid and triglyceride(TG) during autoxidation period.

Identification of hydroperoxides

Fig. 2 and 3 show the typical pattern of IR and NMR spectra of the oxidation products. A single spot identified as monohydroperoxide by TLC showed UV absorption at 235nm specific for conjugated diene structure.

IR spectrum of oxidation products gave absorption at 3450cm^{-1} attributed to the hydroperoxy group, at 988cm^{-1} attributed to *trans-trans* conjugated diene, at 948cm^{-1} attributed to *cis-trans* conjugated diene, at 2800−3000cm^{-1} attributed to C-H streach, at 1465cm^{-1} attributed to C-H bend, at 1741cm^{-1} attributed to ester carbonyl, and at 1166cm^{-1} attributed to C-O. Furthermore, NMR spectrum of oxidation product shows $-(CH_2)_n$ at 1.1~1.8ppm, -CH=CH-CH_2- at 1.8~2.1ppm, conjugated diene at ca, 5.5ppm and hydroperoxy proton at 8.0~8.5ppm. Thus, this compound was identified as monohydroperoxide(MHP).

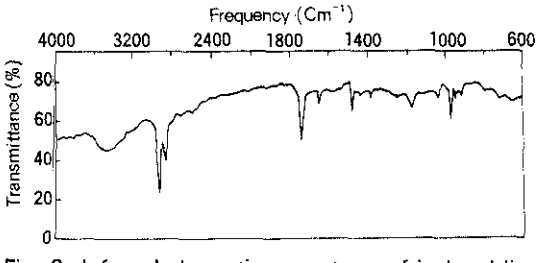

Fig. 2. Infrared absorption spectrum of isolated lipid monohydroperoxide.

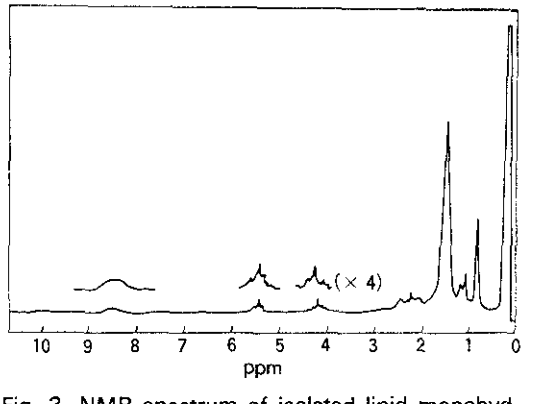

Fig. 3. NMR spectrum of isolated lipid monohydroperoxide.

Autoxidized RAP lipid and oxidized lipid in RAP and PAP also gave a similar TLC, IR and NMR pattern.

Isomeric compositions of hydroperoxide

Table 1 shows the isomeric compositions of hydroperoxy fatty acid components obtained from autoxidized RAP lipid and TG and oxidized lipid in RAP and PAP. Quantitative ratios of the isomers in each compound were in accordance with those of the corresponding fatty acid methyl esters. Hydroperoxy fatty acid components in each compound predominantly consisted of the 9- and 13- hydroperoxide isomers. The 1,4- pentadiene structure in linoleic acid makes them more susceptible to oxidation than the propene system of oleate. The methylene group at position 11 is doubly activated by two adjacent double bonds. Hydrogen abstraction at this position produces a pentadienyl radical intermediate, which upon reaction with molecular oxygen produces an equal mixture of conjugated 9- and 13-diene hydroperoxides[12]. Small amounts of 8-, 10-, 11-, 12- and 16-hydroperoxide isomers were also present.

Isomers of hydroperoxy fatty acid components were formed by autoxidation of each unsaturated fatty acyl group as follows : Oleic acid yielded almost equal amounts of the 8-, 9-, 10- and 11-isomers, and linoleic acid yielded almost equal amounts of the 9- and 13-isomers. Four isomers, the 9-, 12-, 13- and 16- isomers, were obtained from MHP of linolenic acid[9].

The compositions of hydroperoxy fatty acid components obtained from lipid and TG of RAP autoxidized in the dark at 35℃ were similar to those of hydroperoxy fatty acid components of lipid extracted from RAP and PAP stored at 35℃ for four months. It is, therefore, concluded that each unsaturated fatty acyl group is oxidized in a manner similar to fatty acid methyl ester to produce isomeric compositions of hydroperoxides. The contribu-

Table 1. The isomeric composition of methyl hydroxy octadecanoate obtained from raw adlay powder lipid and triglyceride

(Area %)

Sample[a]	POV (meq/kg)	8-OH	9-OH	10-OH	11-OH	12-OH	13-OH	14-OH	15-OH	16-OH
RAP-L	131	4.0	46.7	2.7	2.8	1.6	35.5	ND[b]	ND	6.8
PAP-L	125	4.5	50.7	5.0	5.3	1.0	36.4	ND	ND	5.8
AAL	100	3.4	48.1	2.1	2.6	2.5	36.4	ND	ND	4.9
AAL-TG	120	4.3	47.0	2.9	3.5	2.2	34.2	ND	ND	3.9

[a]RAP-L : raw adlay powder lipid, PAP-L : processed adlay powder lipid, AAL : autoxidized adlay lipid, AAL-TG : autoxidized adlay lipid triglyceride
[b]Not detected

Fig. 4. The proportion of unsaturated fatty acyl group of raw adlay powder lipid and triglyceride to the formation of hydroperoxy fatty acid components.
■ : oleate □ : linoleate ■ : linolenate.

tion of each unsaturated fatty acyl group to the formation of isomeric hydroperoxides was calculated according to the results in Table 1, and was shown in Fig. 4.

According to the calculation, oxidized linolenic acid would yield 56.5% in 12-OH and 16-OH, oxidized oleic acid would yield 50% in 10-OH and 11-OH, so linolenate(Ln)-HPO=(12-OH+16-OH) ÷0.565, oleate(O)-HPO=(10-OH+11-OH)×2 and linoleate-HPO=100-(Ln-HPO+O-HPO)

The analysis of hydroperoxy fatty acid components of oxidized lipid is useful not only in the determination of the proportion of each fatty acyl group to the formation of hydroperoxides, but also in the distinction between the oxidation mechanisms of free radical oxidation. The isomeric compositions of hydroperoxides in oxidized lipid are considered to be related to the rancid odor because most of volatile carbonyl compounds are formed from the isomeric hydroperoxides.

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율무 지방질의 자동산화에 의해서 형성된 Hydroperoxides 이성체 조성

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요 약

생율무가루와 가공된 율무가루를 35℃에서 저장한 시료와, 생율무가루로부터 지방질과 TG를 추출 분획한 후 이들을 35℃에서 자동산화시킨 시료로부터 1차 산화물인 hydroperoxide이성체 조성들을 조사하였다. 자동산화시켰을 때, 이들의 과산화물값이 100에 도달하는 시기는 지방질의 경우는 175, TG의 경우는 80일이 소요되었다. 율무지방질의 1차산화물인 hydroperoxide는 7개의 이성체들로 구성되었으며, 이들중 주요한 이성체들은 9-와 13- hydroperoxide로서 이들은 주로 linoleic acid로부터 산화된 일차생성물이었으며, 이외에 8-, 10-, 11-, 12- 및 16-hydroperoxide 이성체들이 소량 함유되어 있었다. 자동산화된 율무지방질과 TG로부터 얻은 hydroperoxy 지방산 성분들의 이성체 조성들은 저장된 율무가루와 생 율무가루지방질로부터 얻은 것들과 유사하였다.