Autoxidation Rates of Various Esters of Safflower Oil and Linoleic Acid

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Autoxidation rates of five types of safflower oil esters increased in the following order: monoacylglycerol (MG), methyl ester (ME), diacylglycerol (DG), triacylglycerol (TG) and sucrose ester (SE). The differences in autoxidation rate were confirmed by measuring autoxidation of similar esters of linoleic acid. The order of the oxidation rates corresponded to the number of acyl groups per molecule. This relationship was explainable by the idea that intramolecular free radical chain reaction between actyl groups of esters occurs more rapidly than intermolecular chain reaction.

The position of acyl groups also affected the oxidation rates of esters in that 1,3-dilinolein autoxidized more rapidly than 1,2-dilinolein. This difference in oxidation rate may result from a closer arrangement of acyl groups in 1,3-dilinolein than those in the 1,2-isomer.

A few papers have been published on the comparison of the oxidation rates of several types of unsaturated fatty acid esters (1,2). Holman and Elmer (1) reported that the oxidation rates of trilinolein and trilinolenin were higher than those of ethyl linoleate and ethyl linolenate, respectively. However, the reasons for the observed differences in oxidation rates have not been elucidated. Cosgrove et al. (2) studied the kinetics of the autoxidation of polyunsaturated fatty acids and mono-, di- and trilinoleins in chlorobenzene solution. They demonstrated that the oxidation rate/molecule of polyunsaturated fatty acid was directly related to the number of allylic positions present in the molecule, whereas this relationship could not necessarily apply to trilinoleins.

MG and DG are further divided into two positional isomers according to the relative positions of the acyl groups on the glycerol molecule. Accordingly, it is of interest to determine the oxidation rates of these isomers.

This paper reports the differences in the oxidation stabilities of ME, MG, DG, TG and SE. In addition, we examine the oxidation rates of 1,2- and 1,3-DG.

MATERIALS AND METHODS

Materials. Crude safflower oil was extracted from safflower seeds with hexane. Triacylglycerols of safflower oil were prepared from crude safflower oil by silicic acid column chromatography (Kieselgel 60, 30 × 2.5 cm; Merck, Darmstadt, Federal Republic of Germany) with ether/hexane (10:90, v/v) for elution. Methyl esters of safflower oil were obtained by transesterification of TG of safflower oil with 0.5 M sodium methoxide-methanol reagent and purified by silicic acid column chromatography (Kieselgel 60, 30 × 2.5 cm; Merck, Darmstadt, FRG) with ether/hexane (5:95, v/v) for elution.

DG and MG of safflower oil were obtained by transesterification of TG of safflower oil and glycerol (3). The reaction was carried out at 90 C for two hr in the presence of sodium methoxide catalyst. Esters were extracted from the reaction mixture with ether and fractionated on a silicic acid column (Kieselgel 60, 50 × 2.5 cm; Merck, Darmstadt, FRG) eluting with ether/hexane solution. Unreacted TG was eluted with ether/hexane (5:95, v/v). DG and Mg were eluted with ether/hexane (50:50, v/v) and (80:20, v/v), respectively. DG prepared as above were obtained as a mixture of 1,2-isomer (44%) and 1,3-isomer (56%) as determined by TLC-FID. TLC-FID also showed that most of the MG (>99%) was the 1 (or 3)-isomer. The TLC-FID was carried out on boric acid-impregnated silica rods according to Tanaka et al. (4). The instrument used was a TH-10 (Iatron Co. Ltd., Tokyo, Japan) equipped with a Shimadzu R1A integrator (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan). Boric acid-impregnated silica rods were prepared by immersing the Chromarod S-II (Iatron Co. Ltd., Tokyo, Japan) in a 3% boric acid solution. After the rods were activated by passing through the FID scanner, 1 μl of the sample solution containing five μg of esters was applied onto a rod. The rods were developed 10 cm from the origin with acetone/chloroform (96:4, v/v). After developing, the rods were dried in a desiccator for a few minutes and then scanned by the Iatroscan.

Sucrose ester of safflower oil was donated by Mitsubishi Kasei Co. Ltd., Tokyo, Japan. Sucrose has eight available hydroxy groups for esterification. However, the average number of acyl groups/molecule of SE was six. Degree of acylation was determined according to Mima et al. (5).

Fatty acid compositions of the above safflower oil esters were in fairly good agreement with each other (Table 1). They were determined on a Shimadzu GC-6AM gas chromatograph equipped with FID detectors (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan) using a 1.5 m × 3 mm i.d. glass column packed with 10% DEGS on 80/100 mesh Chromosorb W at column temperature 170 C. The detector and injector were held at 230 C. The flow rate of the nitrogen carrier gas was 30

TABLE 1

Fatty Acid Composition of Esters of Safflower Oil

	16:0	18:0	18:1	18:2	18:3
SE	7.5	.8	11.0	78.4	0.3
TG	6.6	2.4	12.4	78.3	0.3
DG	6.6	2.3	13.0	77.9	0.2
MG	6.4	2.1	13.0	78.3	0.2
ME	6.6	2.4	12.4	78.3	0.3

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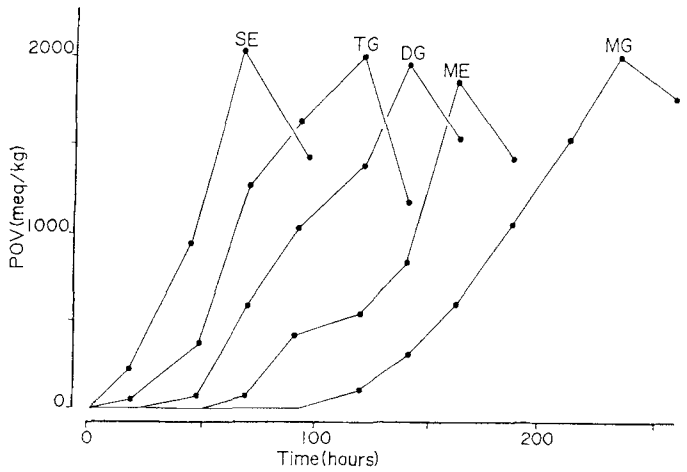

FIG. 1. Changes in POV during autoxidation of esters of safflower oil.

ml/min. Gas chromatographic analyses of MG, DG, TG and SE were carried out after conversion of these esters into their methyl esters with 0.5 M sodium methoxide-methanol reagent and purification by TLC on a 0.5-mm layer of Kieselgel 60 G (Merck, Darmstadt, FRG) by developing with ether/hexane (2:8, v/v).

Di- and trilinoleins were obtained by transesterification of methyl linoleate (ML) and glycerol. The reaction was carried out by the same procedure as that of the preparation of DG of safflower oil. ML was prepared from safflower oil by the method described previously (6). Esters extracted from the reaction mixture with ether were fractionated on silicic acid column (Kieselgel 60, 50 × 2.5 cm; Merck, Darmstadt, FRG) eluting with ether/hexane solution. Unreacted ML was eluted with ether/hexane (5:95, v/v). Tri- and dilinoleins were eluted with ether/hexane (10:90, v/v) and (50:50, v/v), respectively. Dilinolein was further separated into 1,2- and 1,3-dilinoleins on a boric acid-impregnated silica column (Kieselgel 60, 50 × 2.5 cm; Merck, Darmstadt, FRG) eluting with ether/hexane (40:60, v/v) and (50:50, v/v) to separate 1,2- and 1,3-dilinoleins, respectively. Each obtained 1,2- and 1,3-dilinolein fraction contained 6% and 4% of the corresponding isomer, respectively, as determined by TLC-FID. However, no other impurities were detected. TLC-FID conditions are described above.

Oxidation of esters of safflower oil. One g of the esters in a flat-bottomed glass tube (30 ml, 3 cm i.d.) was autoxidized by incubation in the dark at 50 C. Samples for determination of peroxide values (POV) were taken from the oxidized samples at selected time intervals. POV were determined by the colorimetric iodine method (7).

Oxidation of ML, 1,2- and 1,3-dilinolens, and trilinolein. Fifty mg of the ester in a flat-bottomed glass tube (3 ml, 1.3 cm i.d.) was autoxidized by incubation in the dark at 50 C. The autoxidation rate was estimated from changes in the conjugated diene content and determining the unoxidized substrate content during oxidation. The conjugated diene content was determined from the UV absorption data following the AOCS official method (8). The UV absorption was measured in an ethanol solution on a Hitachi 124 spectrophotometer (Hitachi Seisakusho Co., Tokyo, Japan). The quantitative determination of the unoxidized substrate was made on a boric acid-impregnated rod TLC-FID system. The conditions for TLC-FID analysis were the same as described above, except the developing solvent for trilinolein and ML was ether/hexane (20:80, v/v).

RESULTS AND DISCUSSION

The changes in POV of the safflower oil esters during autoxidation are shown in Figure 1. In all cases, POV increased rapidly after an induction period of autoxidation, but then decreased. Judging from the induction period (Table 2), the highest oxidation rate was shown by safflower oil SE, followed by TG, DG, ME and MG. This order agreed with the number of acyl groups/molecule (Table 2). In the propagation stage of autoxidation, fatty alkyl radicals react with molecular oxygen to form peroxy radicals. The peroxy radical abstracts a hydrogen atom from another unsaturated fatty compound to form a hydroperoxide and an alkyl radical. The latter reacts with molecular oxygen in a repetition of the first propagation reaction. The initially formed hydroperoxide may decompose subsequently to yield free radicals such as alkoxy and hydroxy radicals. These radicals serve as initiators for the above reactions (9–11). We suggest that the intramolecular hydrogen abstraction from an unsaturated acyl group by a peroxy radical of another acyl group in an ester molecule occurs more rapidly than the intermolecular hydrogen abstraction, because acyl groups bonded to the same ester molecule are nearer to one another. For this reason, the oxidation rate increased with an increasing number of acyl groups/ester molecule.

The difference in the oxidation rates of ME and MG may be correlated with the following factors: (i) the higher viscosity of MG than ME, and/or (ii) lower

TABLE 2

Comparison of Oxidation Rates of Esters of Safflower Oil

Esters of safflower oil	SE	TG	DG	ME	MG
Acyl groups per molecule	6	3	2	1	1
Time to gain 100 meq/kg of POV	8	25	50	73	120

TABLE 3

Comparison of Oxidation Rates of Esters of Linoleic Acid

Esters of linoleic acid	Time for unoxidized substrate to decrease by 5% (hr)	Time to gain 5% of conjugated diene (hr)
Trilinolein	48	50
1,3-Dilinolein	94	100
1,2-Dilinolein	138	150
ML	194	197

number of double bonds by weight of MG than ME. Sims et al. (12) reported the autoxidation of liquid emulsions containing safflower oil and glycerol, sugars or sugar alcohols. In the study, they demonstrated that the rate of oxygen diffusion decreased as the emulsion viscosities increased. The autoxidation rate also increased with an increasing number of double bonds per weight of lipid (2,11,13).

The oxidation rates of TG, DG and ML of linoleic acid increased in the same order as the safflower oil esters (Table 3). Table 3 also shows that 1,3-dilinolein was autoxidized more rapidly than 1,2-dilinolein. It is known that 1,2-DG and 1,3-DG are isomerized under acidic, basic or thermal conditions to an equilibrium mixture of about 40% of 1,2- and 60% of 1,3-isomer (14). However, little isomerization occurred under the present conditions for incubation, because TLC-FID analysis showed that 1,2- or 1,3-dilinolein was scarcely converted into the corresponding isomer during autoxidation of each single substrate.

Several investigators (15–17) have studied the influence of the position of the unsaturated acyl group on the oxidation rate of TG and demonstrated that acyl groups at the 2-position of glycerol were more resistant to autoxidation than acyl residues at the 1 (or 3)-position. The order of the oxidation rates between 1,2- and 1,3-dilinoleins was consistent with the previous studies (15—17). On the basis of TG structure, Raghuveer and Hammond (15) explained the oxidative stability of the unsaturated acyl group at the 2-position of TG. The difference in the oxidation rates of 1,2- and 1,3-dilinoleins may also be explainable by the conformation of acyl groups in the liquid state of these esters. The higher oxidation rate of 1,3-dilinolein compared to 1,2-isomer could be due to the closer arrangement of the two acyl groups in the 1,3-dilinolein molecule than those in 1,2-dilinolein.

The conformation of 1,2- (18, 19) and 1,3-DG (18,20,21) in solid state is well established. Applegate et al. (19) reported the intramolecular packing arrangement as the lowest-energy conformation of a DG containing a saturated acyl chain in the sn-1 position and a polyenoic fatty acid in the sn-2 position. Larsson (20) and Hyble et al. (21) presented the conformation of 1,3-DG of heavy-atom-substituted fatty acids. In the conformation, the hydrogen-bond system linked the molecules into infinite chains in one direction. On the other hand, the conformation of DG in the liquid state has not been as good, as DG appears to have a certain degree of molecular order. However, molecular shapes in the liquid state will differ from those in solid state. More work is required to elucidate the relationship between the oxidation rates of esters and their conformation in the liquid state.

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