The Effect of Randomization on the Stability of Blends of Trioleoylglycerol and Linseed Oil

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The effect of fatty acid arrangement on triacylglycerols was determined by assessing the stability of mixtures of trioleoylglycerol (consisting of a comparatively stable fatty acid) and linseed oil (containing a high amount of unstable fatty acids in the form of linoleic and linolenic acids) in the ratios of 90:10, 85:15, 80:20, 70:30 and 60:40 (w/w), respectively, before and after enzymatic randomization. Randomization resulted in increased stability; however, increasing the content of the unstable triacylglycerol resulted in a decrease in this effect. Based on these results, it was concluded that randomization of triacylglycerols can have a positive effect on the oxidative stability of an oil if the content of autoxidatively unstable triacylglycerols is low in the original blend. This results in substantial dilution of unstable fatty acids among the more stable triacylglycerols upon randomization.

KEY WORDS: Autoxidation, blended oils, interesterification, linseed oil, randomization, stability, triacylglycerol composition, trioleoylglycerol.

Autoxidation is a reaction whereby atmospheric oxygen is added to unsaturated fatty acids, with the ultimate production of compounds such as shorter-chain alcohols, aldehydes and ketones, as well as high-molecular weight polymers. This reaction has major implications in the food industry, because it causes disagreeable alterations in flavor and texture, as well as production of potentially toxic compounds. Protection against autoxidation is usually sought through special packaging under inert atmospheres (or vacuum) and gas-impermeable barriers, low-temperature storage, hydrogenation or antioxidants, such as butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) or α-tocopherol.

Increasing the autoxidative stability of a fat or oil without the use of these measures would be advantageous to food processors. Alteration of the triacylglycerol composition through rearrangement of the fatty acid moieties has been shown to have an effect on the stability of the resulting product. Numerous studies have shown this effect on the autoxidation of native vegetable oils, with randomization causing a decreased stability in cocoa butter, Borneo tallow, corn and soybean oils (1), lard (2), olive and soybean oils (3), corn oil (4) and soybean oil (5), and increased stability in palm oil (2). However, other published studies have shown no difference in the stability of native and chemically randomized soybean oils (6,7). Recent work suggests that differences regarding the effect of randomization on native vegetable oils may be accounted for by varying methods of stability assessment (8). The same study indicated that the decreased stabilities of randomized oils, as observed to date, may in fact be due to the method of randomization itself, because no differences in stability were found between several native and randomized vegetable oils when enzymatic interesterification was used to obtain random arrangement of the fatty acids (8).

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The theory of protection of an autoxidatively sensitive fatty acid at the middle position of a triacylglycerol (TAG) by stable fatty acids at the two outer positions was first proposed by Raghuveer and Hammond (1), and the authors stated that the effect may decrease as the content of stable fatty acids decreases in the mixture. If this were the case, use of native vegetable oils to investigate this theory is not optimum, because they tend to be comparatively low in stable fatty acids. It would seem that a better means of determining the effect of randomization would be afforded by the use of synthesized TAGs of predetermined stable/unstable fatty acid composition.

There have been several studies that have used either synthetic TAGs or mixtures of pure TAGs with varying ratios of stable/unstable fatty acids. Park *et al.* (7) described the oxidation of synthesized TAGs containing one unsaturated (either linolenic, linoleic or oleic acid) and two saturated fatty acids (palmitic acid), and found no differences in the oxidation rates of TAGs with the same composition but with different arrangements of the fatty acids. On the other hand, Raghuveer and Hammond (1) found that randomized mixtures of trilinoleoylglycerol (1.5%) or trilinoleoylglycerol (1.5%) and trilinolenoylglycerol (0.5%) in tridecanoylglycerol were more stable than simple mixtures of the two compounds, although the effect was less with the latter mixture. Hoffman and coworkers (2) synthesized 13 different TAGs containing palmitoyl, stearoyl, oleoyl and linoleoyl residues at specific positions and found that the stabilities of these oils were not determined solely by the total unsaturation. Wada and Koizumi (5) oxidized both blended and randomly interesterified binary mixtures of equal proportions of a saturated and an unsaturated triacylglycerol, such as tripalmitoylglycerol or tristearoylglycerol with trioleoylglycerol or trilinoleoylglycerol. They observed that the randomized mixtures were significantly more resistant to oxidation than the mixtures of homogeneous TAGs.

Although these studies show that randomization has an effect on the stability of triacylglycerols, no work on the effect of the ratio of stable/unstable fatty acids has been performed, although the paper of Raghuveer and Hammond (1) suggests that this may be important. The present study reports the effect of randomization on triacylglycerols with varying contents of stable to unstable fatty acids. The results may help to explain the effect of randomization and suggest what blends of oils would be effective in utilizing this technique to alter the stability of a commercial product. The commercial use of randomization to impede autoxidation may offer a simple means of increasing the stability of an oil without additives that are unpopular with consumers.

EXPERIMENTAL PROCEDURES

Lipid materials. Linseed oil was used as a source of triacylglycerols with a high content of autoxidatively unstable fatty acids. The cold-pressed oil was a commercial product from Omega Nutrition Inc. (Vancouver, British

Columbia, Canada). Trioleoylglycerol was chosen as a source of a relatively stable fatty acid and was purchased from Sigma Chemical Co. (St. Louis, MO; 95% pure). The choice of a monounsaturated fatty acid over a saturated one was necessitated by the need to keep the lipid liquid and the storage temperature low, because previous research had indicated that detection of stability differences can be obscured when high storage temperatures are used (8,9).

Gas chromatography. Gas-liquid chromatography of methyl esters prepared by acid methanolysis (10) was performed on a Hewlett Packard (Mississauga, Ontario, Canada) Model 5700A gas chromatograph, equipped with a flame ionization detector and a 30-m WB SupelcoWax 10 capillary column (i.d. 0.32 mm) (Supelco, Oakville, Ontario, Canada). Conditions were isothermal, with oven temperature set at 220°C, injector temperature at 200°C and detector temperature at 250°C, with a hydrogen carrier gas flow rate of 1 mL/min. A split ratio of 100:1 was used. Integration was performed by a Hewlett Packard 3392A integrator.

High-performance liquid chromatography. High-performance liquid chromatography (HPLC) was performed on a Beckman Model 342 liquid chromatographic system, equipped with an Ultrasphere ODS-5 μm C-18 (4.6 × 150 mm) column (Beckman Instruments, Inc., Berkeley, CA), and a Supelcosil ODS-5 μm C-18 (4.6 × 250 mm) column (Supelco). Detection was performed with a refractive index detector (Altex Scientific Inc., Berkeley, CA). The mobile phase consisted of a mixture of acetonitrile and tetrahydrofuran (60:40, vol/vol) delivered at a flow rate of 1 mL/min. Sample concentration was approximately 200 mg/mL in chloroform, and a 20-μL injection loop was utilized. For fatty acid analysis of separated peaks, the eluted peaks from four samples were pooled, dried down and then methylated.

Positional analysis. The analysis of the fatty acid composition of the 2-position of purified triacylglycerols was performed *via* a modification of the method of Dutta *et al.* (11). The modifications involved the application of 240 μL of a 200-mg/mL solution of pancreatic lipase in water to the 20 × 20-cm silica gel thin-layer chromatography (TLC) plate, followed by an overlayer of 240 μL of a 100-mg/mL solution of oil in hexane and an incubation time of 10 min at 40°C. The validity of the procedure was checked by comparing the obtained 1,2- and 2,3-diacylglycerol composition with the expected (as calculated from the obtained 2-monoacylglycerol and triacylglycerol compositions and based on a random attack of the pancreatic lipase on the triacylglycerol).

Enzymatic randomization. Enzymatic randomization of trioleoylglycerol and linseed oil was performed by continuously stirring a mixture of oil and 5% (w/w) immobilized nonspecific lipase preparation (SP-382, Novo Laboratories Inc., Copenhagen, Denmark) for 48 h at 60°C under nitrogen in a water-jacketed Wheaton flask. The immobilized enzyme preparation was obtained from Novo Laboratories Inc. and was reported by the company as containing 2% water (wb); this was brought up to a water content of 9.9% (wb) by spraying an appropriate amount of distilled water on the enzyme preparation, then allowing equilibration for 24 h at 4°C.

Experimental method. Trioleoylglycerol and purified linseed oil were blended in the following ratios: 90:10, 85:15, 80:20, 70:30 and 60:40 triolein/linseed (w/w). These blends were each stirred continuously for 30 min, then divided into two portions. One of these was enzymatically randomized. Because peroxides, free fatty acids and mono- and diacylglycerols may effect the stability of an oil (12), and these compounds can be produced through interesterification, all oils were purified prior to storage tests. This purification was performed by column chromatography on alumina (80:200 mesh, Fisher Chemical Co., Edmonton, Alberta, Canada), according to the method of Jensen *et al.* (13). A 2:1 ratio of alumina to oil (w/w) was used, with petroleum ether (b.p. 35–60°C) as eluting solvent. The success of purification was determined *via* TLC on aluminum-backed 0.2-mm silica gel 60 plates that had been activated at 100°C for 1 h, then developed in a solvent consisting of hexane/diethyl ether/acetic acid (50:50:0.5). Upon removal from the TLC tank, the plate was air-dried, then exposed to iodine vapor for 5 min to allow detection of nontriacylglycerol materials. To maintain consistent experimental conditions, the blended oils were purified in the same manner.

All samples were subjected to fatty acid and positional analyses. In addition, a profile of the TAG composition was obtained *via* HPLC. Aliquots of each oil were distributed in 0.5-mL amounts in 4-mL vials and stored at 52°C. At appropriate intervals, a vial of each was removed and the absorbance at 234 nm determined in duplicate as indicative of conjugated diene content (14).

RESULTS AND DISCUSSION

The results of positional analyses of mixed and randomized trioleoylglycerol/linseed blends of varying ratios (blending period of 30 min, randomization period of 48 h) are presented in Table 1. A comparison of the calculated and the obtained diacylglycerol compositions within each sample supports the validity of the method used for positional analysis (*i.e.,* the attack of the pancreatic lipase was, in fact, random and did not show selectivity towards any specific fatty acids). In addition, the similar values obtained for the fatty acid compositions of the TAGs and the corresponding 2-positions of each randomized blend reveal the success of the randomization treatment. Finally, in all but the oils possessing the highest ratio of trioleoylglycerol/linseed oil, the fatty acid compositions of the total TAG of the mixed and randomized oils were similar, an important characteristic when comparing stabilities of the two oils. However, in the trioleoylglycerol/linseed oil (90:10, w/w), the randomized oil possessed 55% more linolenic acid than the mixed oil. This can be accounted for by the purification procedure that commonly results in the loss of some TAGs on the alumina column. Because the more highly unsaturated TAGs are expected to be retained to a slightly greater degree on the polar alumina column, specific loss of TAGs containing linolenic acid is not unexpected. Due to the low content of this fatty acid in the original mixture, the loss of a small amount during purification results in a decrease of a large proportion of the original amount of linolenic acid.

The extent to which the triacylglycerols had been rearranged can be clearly seen in Figures 1–4, which depict high-performance liquid chromatograms of the mixed and randomized blends of several ratios. The increase in the proportion of linseed oil in the mixed and randomized

TABLE 1

Fatty Acid Composition of Fractions Resulting from Positional Analyses of Mixed and Randomized Trioleoylglycerol/Linseed Blends of Varying Ratios (mixed for 30 min; randomized for 48 h)

Ratio (trioleoylglycerol/linseed oil, w/w)	Fatty acid (mole %)				
	16:0	18:0	18:1	18:2	18:3
90:10 Mixed					
Triacylglycerol	1.1	0.3	94.2	0.7	3.8
sn-2 Monoacylglycerol	2.0	0.4	89.7	2.1	5.2
Calculated diacylglycerol	1.3	0.3	93.1	1.0	4.1
Obtained diacylglycerol	15.	0.3	92.6	12.2	4.1
90:10 Randomized					
Triacylglycerol	1.2	0.3	91.3	1.3	5.9
sn-2 Monoacylglycerol	2.1	0.6	88.6	1.6	6.7
Calculated diacylglycerol	1.5	0.4	90.7	1.4	6.1
Obtained diacylglycerol	1.7	0.5	90.6	1.5	5.7
85:15 Mixed					
Triacylglycerol	1.5	0.4	89.1	1.4	7.7
sn-2 Monoacylglycerol	1.4	0	86.5	3.4	9.0
Calculated diacylglycerol	1.4	0.3	88.5	1.8	8.0
Obtained diacylglycerol	1.7	0.5	88.7	2.0	6.9
85:15 Randomized					
Triacylglycerol	1.5	0.5	88.0	1.8	8.3
sn-2 Monoacylglycerol	2.1	0.6	86.0	1.8	8.3
Calculated diacylglycerol	1.7	0.5	87.5	1.9	8.4
Obtained diacylglycerol	1.8	0.6	87.5	2.0	8.0
80:20 Mixed					
Triacylglycerol	1.7	0.6	85.1	2.5	10.2
sn-2 Monoacylglycerol	1.6	0.1	80.4	4.3	13.0
Calculated diacylglycerol	1.7	0.5	83.9	2.9	10.9
Obtained diacylglycerol	2.1	0.6	82.3	3.3	10.9
80:20 Randomized					
Triacylglycerol	1.9	0.6	83.7	3.1	10.7
sn-2 Monoacylglycerol	3.4	0.9	80.6	3.1	11.3
Calculated diacylglycerol	2.3	0.7	83.0	3.1	10.8
Obtained diacylglycerol	2.6	0.9	82.3	3.1	10.9
70:30 Mixed					
Triacylglycerol	2.2	0.9	77.3	4.1	15.6
sn-2 Monoacylglycerol	2.3	0.6	69.8	6.5	20.2
Calculated diacylglycerol	2.2	0.8	75.4	4.7	16.7
Obtained diacylglycerol	2.5	0.9	74.4	5.0	17.0
70:30 Randomized					
Triacylglycerol	2.4	1.1	74.7	4.4	17.5
sn-2 Monoacylglycerol	3.8	1.4	71.8	4.6	17.9
Calculated diacylglycerol	2.8	1.2	74.0	4.4	17.6
Obtained Diacylglycerol	2.7	1.2	74.2	4.7	17.1
60:40 Mixed					
Triacyglycerol	2.9	1.2	68.3	5.6	22.0
sn-2 Monoacylglycerol	1.3	0.3	62.1	8.6	27.5
Calculated diacylglycerol	2.5	1.0	66.8	6.3	23.4
Obtained diacylglycerol	2.7	1.2	64.8	6.6	24.6
60:40 Randomized					
Triacyglycerol	2.8	1.3	66.6	5.7	23.6
sn-2 Monoacylglycerol	3.6	1.7	63.1	6.4	25.0
Calculated diacylglycerol	3.0	1.4	65.7	5.8	24.0
Obtained diacylglycerol	3.2	1.7	65.2	6.1	23.6

samples can be seen readily in the increase in the peaks 1 through 5 in the chromatograms of the oils. The change that occurred in TAG composition with randomization is clear with a loss of the first two peaks in the 90:10 mix and an apparent general decrease in complexity in all of the ratios tested.

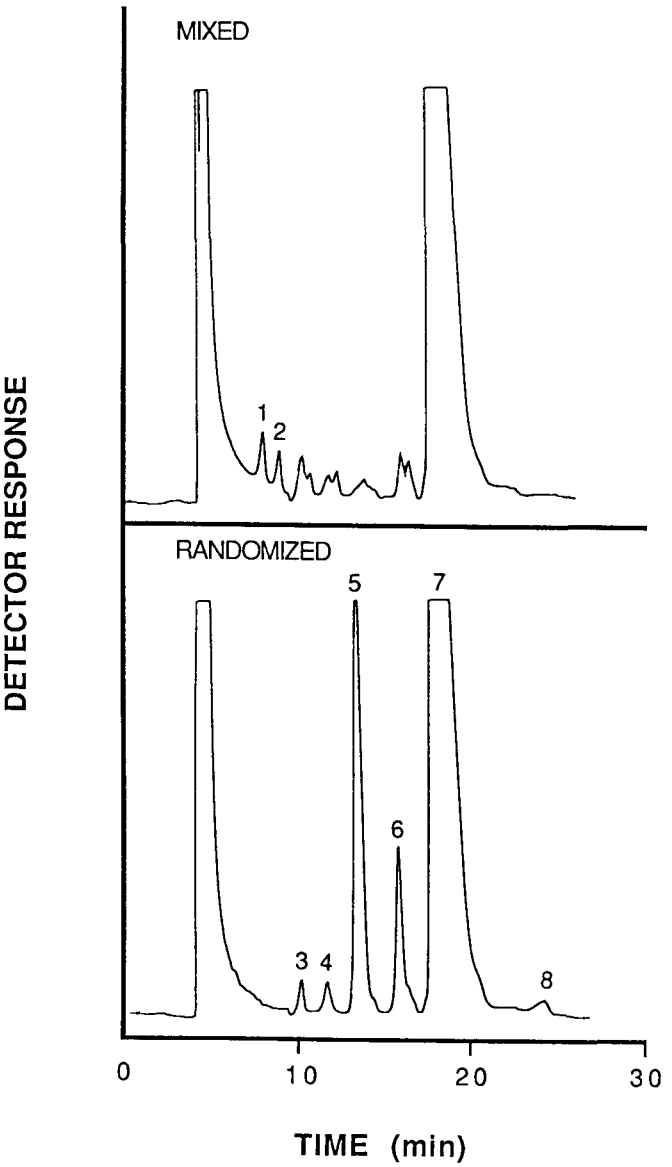

FIG. 1. High-performance liquid chromatograms of mixed and randomized trioleoylglycerol/linseed oil (90:10, w/w) blends.

Table 2 lists the results of fatty acid analyses of the peaks collected from the 60:40 randomized and mixed blends and the 80:20 randomized blend. These samples were chosen for this analysis to determine what changes would occur in the fatty acid composition of each peak with randomization. Such information also would show whether randomized blends of different ratios would exhibit different fatty acid compositions for peaks of the same equivalent carbon number (ECN). No difference in the fatty acid composition of the peaks from one mixed blend to another was expected.

The first peak in the analysis of the trioleoylglycerol/linseed oil (60:40, w/w) mixture was composed mainly of 18:3, and most likely represented trilinolenoylglycerol, which matches with an ECN of 36. Likewise, with a rough ratio of 1:2 of 18:2 to 18:3, peak 2 was composed mainly of linoleoyldilinolenoylglycerol, which matches an ECN of 38. The next three peaks appear to be a combination

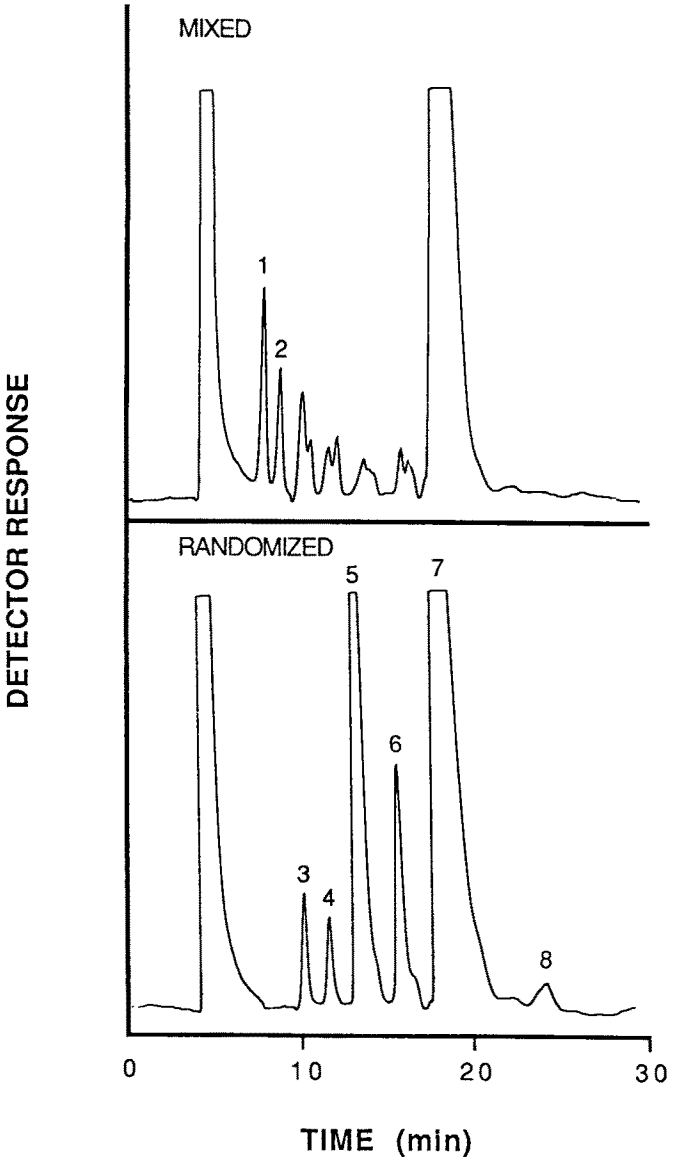

FIG. 2. High-performance liquid chromatograms of mixed and randomized trioleoylglycerol/linseed oil (80:20, w/w) blends.

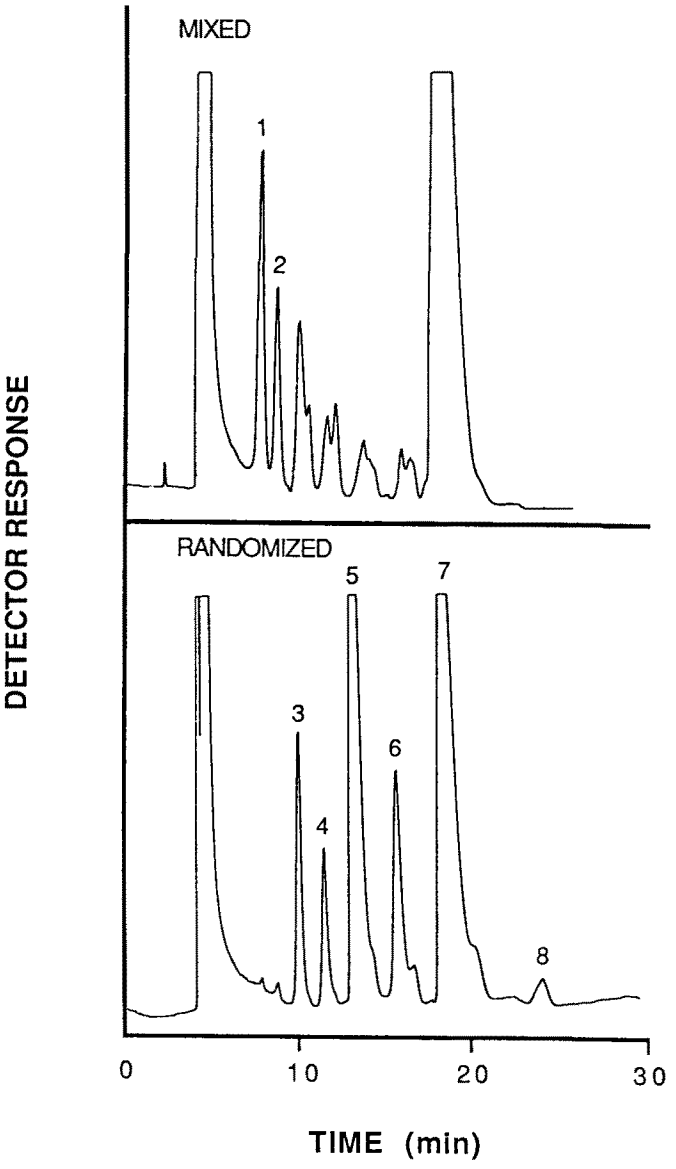

FIG. 3. High-performance liquid chromatograms of mixed and randomized trioleoylglycerol/linseed oil (70:30, w/w) blends.

of several different TAGs, none of which was present in sufficient quantities to produce clear-cut ratios of fatty acids. However, if ECNs of 40–44 are assigned, the sixth peak would then have an ECN of 46, which matches a composition of linoleoyldioleoylglycerol. Peak 7 was analyzed as consisting almost completely of oleic acid, which matches an ECN of 48. The final peak must have contained some stearoyldioleoylglycerol, as represented by the presence of stearic and oleic acids and an ECN of 50 (the higher content of oleic acid obtained than would have been expected may have been due to contamination through the procedure used for collecting and pooling the individual peaks).

The values for the 60:40 randomized oil shown in Table 2 indicate that upon randomization, the composition of the first two peaks remained the same. However, the composition of peak 3 shows a much greater increase in oleic acid (probably in the form of oleoyldilinolenoylglycerol), as does peak 4 (probably in the form of oleoyllinoleoyllinolenoylglycerol) and peak 5 (probably in the form of linolenoyldioleoylglycerol). A large increase in only one type of triacylglycerol would explain the appearance of clean undivided peaks in the HPLC chromatogram of the randomized oil. The increase in these particular TAGs reveals the effective spread of the fatty acids originally located on the linseed oil triacylglycerols throughout the trioleoylglycerols in randomized mixes initially possessing a high proportion of the latter. The major TAGs under peaks 7 and 8 remained as trioleoylglycerol and stearoyldioleoylglycerol, respectively.

Comparison of the fatty acid analyses of the peaks representing the 60:40 and the 80:20 trioleoylglycerol/linseed oil randomized mixtures reveal little change in the content of fatty acids beneath each peak (although there was a loss of the first two peaks with randomization of the latter ratio), suggesting that the composition of each

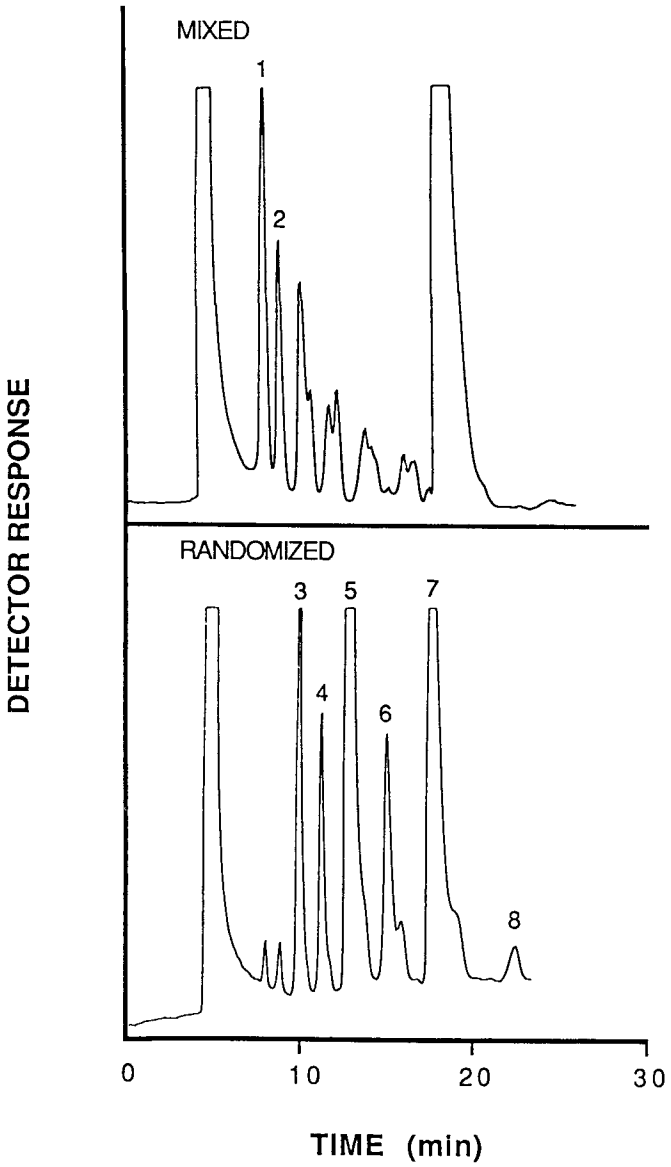

FIG. 4. High-performance liquid chromatograms of mixed and randomized trioleoylglycerol/linseed oil (60:40, w/w) blends.

peak resulting from randomization remains more or less the same, and only the proportion of each species changes with the changing ratios of trioleoylglycerol/linseed oil in the original blend.

Table 3 provides an overview of the changes in the proportions of individual TAG species that made up the mixed and randomized oils of the ratios of trioleoylglycerol/linseed oil used. It is apparent that randomization was effective at spreading the linolenic acid residues throughout the bulk of the oil, as the content of trilinolenoylglycerol (peak 1), linoleoyldilinolenoylglycerol (peak 2) and oleoyldilinolenoylglycerol (peak 3) decreased substantially in the randomized mixtures, while the increasing content of oleic acid within each species resulted in the overall increase in peaks representing TAGs containing two oleic acid residues (peaks 5, 6 and 8), with linolenoyldioleoylglycerol (peak 5) experiencing the largest increase (due to the original high percentage of linolenic acid in the linseed oil). It was only at the level of 40% (w/w) linseed oil that the contents of oleoyldilinoleoylglycerol (peak 3) and oleoyllinoleoyllinolenoylglycerol (peak 4) were seen to increase, due to the higher content of linseed triacylglycerols preventing the complete distribution within the trioleoylglycerol.

TABLE 2

Fatty Acid Composition of Peaks Collected from HPLC of Two Randomized and One Mixed Blend of Trioleoylglycerol/Linseed Oil (w/w)

Sample	Peak no.	Fatty acid (mole %) 16:0	18:0	18:1	18:2	18:3	Probable ECN[a]
60:40 Mixed	1	0	0	0.5	0	99.5	36
	2	0.2	0	0.7	29.8	69.4	38
	3	7.1	0	16.0	14.3	62.6	40
	4	6.2	12.8	13.1	24.2	43.8	42
	5	7.1	6.8	37.7	18.7	29.6	44
	6	3.6	8.8	54.2	24.3	9.1	46
	7	0.2	0	99.8	0	9	48
	8	4.4	13.2	82.4	0	0	50
60:40 Randomized	1	1.8	0	6.5	0	91.7	36
	2	0.8	0	5.4	30.5	63.2	38
	3	0.2	0	29.2	1.6	69.1	40
	4	0.5	1.5	29.8	32.7	35.5	42
	5	2.1	0.1	63.6	1.4	32.8	44
	6	1.8	3.4	60.4	29.6	4.7	46
	7	2.9	0.3	96.8	0	0	48
	8	1.2	28.3	70.5	0	0	50
80:20 Randomized	3	0	0	36.3	0	63.7	40
	4	1.5	0	37.0	28.5	33.0	42
	5	1.1	0	67.2	0.2	31.6	44
	6	1.0	1.4	70.0	26.7	1.1	46
	7	1.8	0	98.2	0	0	48
	8	1.3	25.6	73.1	0	0	50

[a]ECN, equivalent carbon number.

Figure 5 presents the absorbance at 234 nm for each of the randomized and mixed oil blends taken throughout storage at 52°C. Both a shortened induction period and an increase in slope are seen for samples containing higher percentages of linolenic acid. This relationship is not unexpected, considering that differences in the stability of oils that possess increasing contents of unsaturated fatty acids has been observed previously (9,15).

The second effect, revealed by Figure 5, is the influence of the randomization treatment, which brings about dilution of linolenic acid among triacylglycerols containing predominantly oleic acid. Within each randomized and mixed blend, with the exception of the 90:10 ratio, the randomized oil consistently exhibited a longer induction period. The apparent discrepancy shown by the curves representing the 90:10 ratio can be attributed to the sizeable difference in linolenic acid content. As seen in Table 1, the content of this compound in the mix is only 64% of that present in the randomized blend. When the concentration of linolenic acid is as small as it is in the 90:10 randomized and mixed blends, any variations in content can be expected to result in larger differences in autoxidative susceptibility than would be the case in samples possessing much larger proportions of the fatty acid.

An additional, important observation is the decrease in the difference in the evident stabilities of the randomized

TABLE 3

Percent Areas of Peaks from HPLC of Mixed and Randomized Trioleoylglycerol/Linseed Oil Blends of Various Ratios

Trioleoylglycerol/linseed oil ratio (w/w)	Treatment	Peak no. 1 (36)[a]	2 (38)	3 (40)	4 (42)	5 (44)	6 (46)	7 (48)	8 (50)
90:10	Mixed	1.1	0.9	2.1	1.4	1.1	2.4	90.9	0.3
	Randomized	0	0	0.9	1.1	15.1	5.0	77.6	0.5
80:20	Mixed	4.3	3.1	4.5	3.2	1.5	2.7	80.3	0.4
	Randomized	0	0	2.3	2.0	25.0	7.0	62.5	1.3
70:30	Mixed	6.8	4.5	6.8	4.6	2.1	2.4	72.7	0.1
	Randomized	0.1	0.3	6.3	4.0	34.4	9.1	44.2	1.6
60:40	Mixed	9.5	5.9	8.5	5.5	3.4	2.7	63.7	0.3
	Randomized	0.7	0.9	11.7	6.4	37.0	9.6	32.9	0.9

[a]Probable ECN.

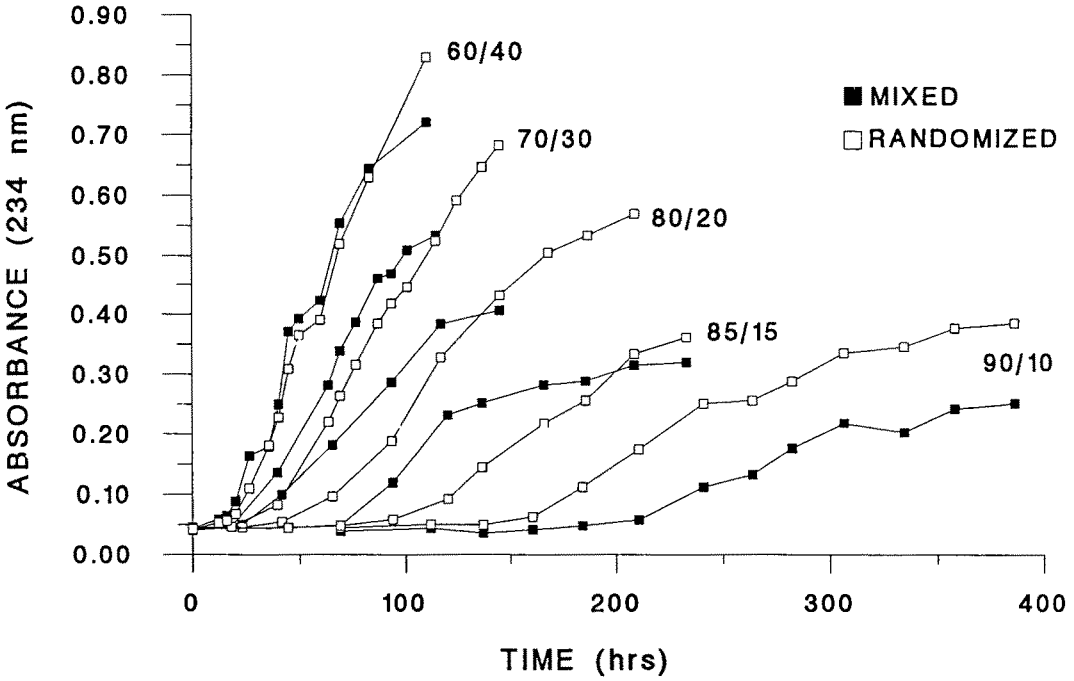

FIG. 5. Absorbance at 234 nm (indicative of conjugated diene content) of mixed and randomized trioleoylglycerol/linseed oil blends of various ratios (w/w), as a function of storage time at 52°C.

and mixed blends with a decrease in the trioleoylglycerol/linseed oil ratio; *i.e.*, as the content of linseed oil increases in the blends, the effect of randomization decreases.

The differences in induction periods in Figure 5 are clear, and when these are plotted against percentage of linolenic acid, the relationship can be seen in Figure 6. The resultant curves reveal a rapid decrease in the induction periods of both blended and randomized oils up to a linolenic acid content of 10 mole%. Successive increases in linolenic acid content above this level result in small changes in the induction period. Also apparent from these curves is the greater stability of the randomized oils. Finally, Figure 6 reveals the decreasing difference between the induction periods of mixed and randomized blends as the linolenic acid content is increased, particularly in the oils containing more than 10 mole% of linolenic acid.

The stabilizing effect of randomization observed when the ratio of unstable to stable triacylglycerols is low can be explained by dilution of the sensitive fatty acids, so that they no longer exist on the same triacylglycerol but are distributed throughout the bulk of the oil. Since autoxidation is a free-radical reaction, the rate must depend upon collision of free radicals with sensitive fatty acids. If these are in close proximity, the rate can be expected to be higher than when they are separated and surrounded

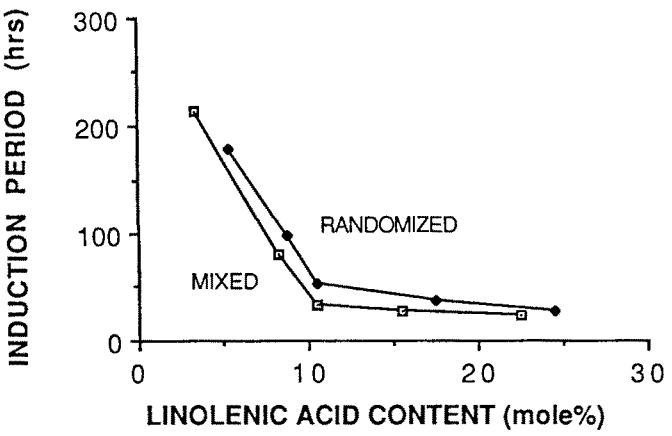

FIG. 6. Length of induction period at 52°C of mixed and randomized trioleoylglycerol/linseed oils of varying ratios, as a function of linolenic acid content (mole%).

by relatively stable fatty acids. Thus, the decrease in the effectiveness of randomization that was observed when the proportion of sensitive TAGs increased within the mixture is explainable by a reduction in the amount of separation achievable through randomization to the point that even with randomization, there were unstable fatty acids existing on the same triacylglycerol. At this point, the effect can be expected to be small, if present at all.

This hypothesis is a simpler one than that previously put forth, which supposes the protection of sensitive fatty acids located at the 2-position by stable fatty acids located at 1 and 3. The latter hypothesis requires at least a somewhat rigid structure of triacylglycerols, which has never been shown in liquid oils. However, the dilution hypothesis requires no formal structure, and yet can be imagined to exist in a system possessing any degree of semi-permanent arrangement.

These results indicate that randomization of two homogeneous triacylglycerols, as performed by several other researchers (1,2,5), may not necessarily have shown protection of the 2-position by stable fatty acids present at the 1- and 3-positions, but were more likely caused by dilution of the autoxidatively sensitive fatty acid molecules. The results obtained when the concentration of the sensitive fatty acid was increased past 10% in the present study support this hypothesis of dilution, because with randomization there would always be twice as many 1,2-stable, 3-unstable triacylglycerols produced as 1,3-stable, 2-unstable; the appearance of a significant effect was observed only when the concentration of unstable fatty acids was less than a critical level. That this level may vary depending upon the fatty acids used is shown by the work of Wada and Koizumi (5), who obtained greater stability with randomization of 50:50 (w/w) mixtures of a fully saturated fatty acid and trioleoyl or trilinoleoylglycerol.

The major conclusion reached from these experiments is that randomization of an oil containing a high amount of unstable fatty acids with one consisting of a large proportion of stable fatty acids can have an effect on the ultimate stability. However, this is affected by the initial ratios of the two homogeneous triacylglycerols. Increasing the content of the unstable triacylglycerol results in a decrease in the decelerating effect of randomization.

The implications are of practical importance to the fats and oils industry. It is now known that randomization makes it possible to obtain a mixed oil of stable and unstable triacylglycerols that is more resistant to autoxidation than would be possible by merely blending the two. This is achieved through two effects: i) randomization causes the dilution of sensitive fatty acids throughout the bulk of the oil; and ii) randomization causes a decrease in the melting point of a blend, thus allowing for the inclusion of a greater percentage of a higher-melting stable fatty acid (assuming a liquid to plastic product is desirable). In this manner, a stable product may be achievable that still has a reasonably high content of an unstable fatty acid, which may have nutritional benefits.

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