Effect of Randomization on the Oxidative Stability of Corn Oil

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ABSTRACT: Randomized corn oil TAG oxidized much faster than natural oil, but after purification with alumina, they oxidized at the same rate. We showed that this effect could not be attributed to a difference in total tocopherols in the randomized and natural oils. Polar material recovered from the alumina treatment was fractionated by TLC, and a pro-oxidant effect was found in the fractions containing MAG and DAG. However, MAG and DAG, although mild pro-oxidants, could not account for the pro-oxidant effect generated by randomization. No other compounds could be detected in the MAG fraction by MS. The pro-oxidant effect of randomized oil disappeared when EDTA or citric acid was added in sufficient amounts. The pro-oxidant effect of randomized corn oil was increased by the incorporation of additional copper or iron at a concentration that did not catalyze oxidation of the purified oil. Treatment of corn oil with ascorbic acid, ascorbyl-6-palmitate, ethyl acetoacetate, ethyl diacetoacetate, and acetylacetone did not reproduce the effect of the unknown pro-oxidant. Although the identity of the pro-oxidant is still unknown, we have confirmed that it is produced during randomization; it does not have pro-oxidant activity alone, but it facilitates the catalytic activity of the transition metal ions.

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KEY WORDS: Chelating agents, corn oil, interesterification, oxidation, purification, randomization, transition metal ions.

The rate of oxidation of randomized TAG from soybean and corn oil was reported to be three to four times faster than that for natural TAG (1,2). Raghuveer and Hammond (3) first reported that the rate of oxidation of physical mixtures of unsaturated TAG (1.5–2%) and tridecanoin was dramatically decreased after interesterification. This change in oxidation rate was attributed to dispersion of the unsaturated FA in the TAG molecules. They also showed that randomization of several natural fats increased their oxidation rate. Later studies confirmed the effect of randomization on stabilizing mixtures of trisaturated and unsaturated TAG oxidized at 50 (4) and 180°C (5). But Neff *et al*. (6) showed that no preferential oxidation occurred among the acyl groups on the *sn*-1,3 and *sn*-2 positions of glycerol. Research by Park *et al*. (7–9) also showed no difference in the rates of oxidation (at 37 or 50°C) before and after randomization of synthesized TAG or TAG from purified soybean oil. They concluded that any possible increase of the oxidation rate of randomized TAG was caused by a depletion of tocopherols. These results suggest that different experimental conditions, especially the presence or absence of oil purification, may play an important role in the effect of randomization on TAG oxidative stability. We wanted to verify and investigate the effects reported by Lau *et al*. (2), but our failure to verify these data led us to a study of the cause of the increased oxidation rate in randomized oils.

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MATERIALS AND METHODS

Two brands of corn oil (Elite, Bunge Foods, Bradley, IL; Vital, ACH Food Companies, Inc., Memphis, TN) were purchased locally. Palmitic acid, 1-monolinoleoyl-*rac*-glycerol, and 1,3-dilinoleoyl-*rac*-glycerol were from Nu-Chek-Prep (Elysian, MN). Acetylacetone, ethyl acetoacetate, ethyl diacetoacetate, and porcine pancreatic lipase (EC 3.1.1.3) were purchased from Sigma-Aldrich (St. Louis, MO). Other reagents and solvents were from Fisher Scientific (Pittsburgh, PA). Diethyl ether was purified by distillation from lithium aluminum hydride to remove peroxides and phenolic antioxidants that had been added as stabilizer. It was stored at 4°C for no more than a week before use.

Oil randomization. Corn oil was randomized with 0.5% sodium methoxide at 60–80°C for 1 h under vacuum. Acetic acid was added to terminate the reaction, and the oil was washed with warm water (35–40°C) until the water was neutral, dried with anhydrous sodium sulfate, and centrifuged (IEC Centra MP4; International Equipment Company, Needham Heights, MA) at 1430 × *g* for 5 min to obtain a clear oil.

Randomization was confirmed by comparing the *sn*-2 FA composition of the original and randomized oil using the method of Luddy *et al*. (10). About 50 mg of oil was treated with pancreatic lipase; the 2-MAG were isolated by TLC, converted to methyl esters, and analyzed by GC. For GC, a capillary column (15 m × 0.25 mm, 0.2 μm, Supelco SP-2330; Supelco, Bellefonte, PA) was used at 190°C in a Hewlett-Packard 5890 Series gas chromatograph. The temperature of the detector and injector was 230°C, and the carrier gas was helium at a flow rate of 1 mL/s.

Oil purification. Oil dissolved in hexane was passed through an alumina column (either 2.4 cm i.d. × 30 cm length or 1.4 cm i.d. × 18 cm length) to remove polar impurities and tocopherols according to the method of Jensen *et al.* (11). Alumina was

activated by heating to 260°C overnight, and oil was passed through the alumina column at a ratio of oil to alumina of 1:2 by weight. TAG were eluted with 3 column vol of 5% diethyl ether in hexane. More polar compounds were eluted with 3 column vol of methanol. At times, the column polar extract was further concentrated by a second alumina purification step to obtain a highly concentrated polar fraction for TLC separation. The TLC fractionation was done on an Alltech Adsorbosil-Plus 1 (Alltech, Deerfield, IL) 20 × 20 cm plate with 500 μm thickness, using hexane/ether/acetic acid (60:40:2, by vol) as a developing solvent.

Oxidation rate determination. Five-gram oil samples were oxidized in 125-mL Erlenmeyer flasks at various temperatures in an incubator in the dark. Samples were taken periodically, and the PV were measured according to the ferrous iron oxidation method (12). An oil sample of 50–400 mg was weighed accurately into a 10-mL volumetric flask and diluted with ethanol/benzene (80:20). Next, 50 μL ammonium thiocyanate solution (3.75 M) and 100 μL ferrous chloride solution (approx. 0.014 M) were added to the flask and mixed well. After 10 min, the absorbance was measured at 515 nm with a Hitachi U2000 spectrophotometer (Tokyo, Japan), and the PV was calculated based on a standard curve. The standard curve was established as a linear plot of the absorbance of a series of dilutions of an oxidized oil sample against the microequivalents of hydroperoxide in each tube. The PV of the original oxidized oil was determined by using AOCS Official Method Cd 8-53 (13). The oxidation rate constant was determined from the slope of a plot of the natural logarithm of the PV (as determined by the ferrous ion method) vs. time (14).

Oxidative stability test. Oxidative stability was measured as an index (OSI value, in h) using the ADM Oxidative Stability Instrument (Omnion, Rockland, MA) at a specific temperature, according to AOCS Official Method Ca 5a-40 (13). All determinations were in duplicate.

Quantification of MAG and DAG in corn oil. MAG and DAG contents in corn oils were analyzed by HPLC according to the method of Liu *et al*. (15) on a Beckman Coulter HPLC with an autosampler 508 and solvent delivery module 126. An ELSD (ELSD 2000; Alltech) was used with the drift tube temperature set at 55°C and the carrier gas (nitrogen) flow rate at 1.6 L/min. A Chromegasphere SI-60 column (150 × 3.2 mm, 5 μm; ES Industries, Marlton, NJ) was used for separation. The mobile phase was: channel A, hexane; channel B, hexane/2-propanol/ethyl acetate/10% formic acid (80:10:10:1, by vol), and the flow rate was 0.7 mL/min. The amounts of MAG and DAG were based on a standard curve of detector response vs. mass.

HPLC analysis of tocopherol. Tocopherols were analyzed according to AOCS Official Method Ce 8-89 (13).

GC–MS characterization of MAG extract from the TLC plate. The MAG band separated by TLC of the polar extract from an alumina column was extracted from the silica with methanol. The MAG extract was analyzed by GC–MS using a Micromass GCT mass spectrometer (Micromass, Beverly, MA). The sample was separated by a DB-5 MS column (30 m × 0.25 mm, 0.25 μm; J&W Scientific, Folsom, CA) in an Agilent 6890 Series GC System (Agilent Technologies, Palo Alto, CA) with the oven temperature programmed from 150 (1 min) to 300°C (5 min) at 15°C/min rate, injector temperature at 260°C, helium carrier gas at 1 mL/s, and injector split ratio of 25:1. The MS condition was electron impact ionization with the scan cycle at 0.75 s and mass range from 45 to 500.

Statistical analysis. Statistical analyses were performed using PC SAS (SAS Institute, Cary, NC) software using a general linear module (16).

RESULTS AND DISCUSSION

Effect of randomization on oxidation rate of the purified oil. Lipid oxidation curves of the alumina-purified natural and randomized corn oils are shown in Figure 1. When converting the PV values to their natural logarithm and plotting these values against time (in h), we typically obtain straight lines, and the slopes of the lines are considered the oxidation rates. Oxidation rates of six lots of natural and randomized corn oils after purification are shown in Table 1. Statistical analysis showed that there was no difference in the oxidation rate of natural and randomized corn oil after purification ($P = 0.136$). However, there was a significant difference among lots of oils ($P < 0.0001$). The factors causing the differences in oxidation rate among lots were not characterized, although the FA compositions of the oils were determined to be very similar.

To be certain that the randomization reaction was complete, the FA composition at the *sn*-2 position of the randomized oil was compared with the overall composition of the natural TAG and the composition at the *sn*-2 position of the natural oil (Table 2). The nearly identical composition of the *sn*-2 FA of the randomized oil and of the natural oil TAG was an indication of satisfactory randomization. These results indicate that the alteration of FA distribution by randomization does not affect the oxidation rate of corn oil.

Previous work (1,2) suggesting that randomized corn oil oxidized 3–4 times faster than its natural oil could not be confirmed,

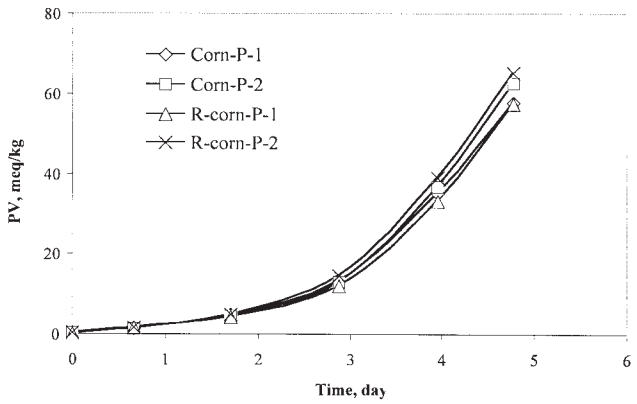

FIG. 1. Effect of randomization on oxidation at 28°C of replicate corn oils. Corn-P is an alumina-purified natural corn oil; R-corn-P is an alumina-purified randomized corn oil.

TABLE 1
Oxidation (at 28°C) Rates of Natural and Randomized Corn Oils After Alumina Purification[a]

Oil	Natural	Randomized	Main effect
Lot 1	0.90	0.91	0.91
Lot 2	0.79	0.92	0.83
Lot 3	0.93	0.80	0.86
Lot 4	1.08	1.11	1.10
Lot 5	1.26	1.19	1.22
Lot 6	1.29	1.24	1.27
Main effect	1.01	1.01	

[a]The $LSD_{0.05}$ values for comparing lot of oil and randomization treatment are 0.06 and 0.03, respectively.

TABLE 3
Oxidative Stability (h, at 100°C) of Four Lots of Natural and Randomized Corn Oils by the OSI Method Without Alumina Purification[a]

Oil	Natural	Randomized
Lot 1	18.93	10.67
Lot 2	20.70	3.60
Lot 3	20.80	5.73
Lot 4	20.08	9.45
Main effect	20.13	7.73

[a]The $LSD_{0.05}$ values for comparing different lots of oil and randomization of oil are 0.31 and 0.22, respectively. OSI, oxidative stability index.

even though similar purification procedures with alumina columns were used in these studies. We evaluated the effectiveness of the alumina purification by measuring the residual tocopherols in the oils. Our HPLC analyses showed that at least 99% of the tocopherols were removed by the alumina purification, and we assume other relatively polar impurities also were removed. Lau *et al*. (2) also reported that their alumina treatment removed tocopherol.

Concluding that randomization of the TAG or acyl distribution on TAG has no effect on oxidation rate may be premature. Hoffmann *et al*. (17) examined a variety of synthetic TAG and reported their oxidation rates as measured by the consumption of oxygen by the fat at 85°C. They found that 1,3-equiacyl TAG (glyceryl 2-oleate-1,3-distearate) tended to be much more stable than its 1,2-equiacyl isomer (glyceryl 1-oleate-2,3-distearate) during lipid oxidation. However, different distributions of glyceryl oleate dilinoleates (glyceryl 1-oleate-2,3-linoleate vs. 2-oleate-1,3-linoleate) did not show any difference in oxidation rate. In our study, the greater number of fatty acyl types (5, compared with 2 or 3 in the pure compounds) and the greater degree of unsaturation may have masked the effect of acyl group distribution on oxidation. The studies of Wada and Koizumi (4) and Yoshida and Alexander (5) confirmed the findings of Raghuveer and Hammond (3) for the randomization of mixtures of trisaturated and triunsaturated TAG.

Effect of randomization on oxidative stability of the unpurified oil. The oxidative stability (OSI) (h, at 100°C) of four different samples of natural and randomized corn oils without purification is shown in Table 3. The data show a significant decrease in stability of the randomized corn oil. We also found that the OSI times of randomized oils differed among various lots of oil, ranging from 3.6 to about 10.7 h. Since these were unpurified oils, any change in concentration of tocopherol or unknowns during randomization of oil could affect the oxidation rate. Other minor compositional or reaction differences also could potentially affect oxidation rate. Quantification of tocopherols by HPLC showed a 9.7% reduction of tocopherols in the randomized oil (Table 4). The reduction in total tocopherol alone in the randomized oil did not account for the increased oxidation rate as shown by a test model in which tocopherols were added to a purified corn oil at 100 (913 ppm) and 90% of the level found in natural oil (25% α-, 72% γ-, and 3% δ-tocopherols); the OSI (at 100°C) times were 14.6 and 14.4 h, respectively. When tocopherols were added at 60 and 30% of the original level, the OSI values were reduced to 13.2 and 10.5 h, respectively. These results suggest that the increased oxidation rate in the randomized corn oil was not caused by the 9.7% reduction in tocopherol content. These results also seem to show that randomization has produced a pro-oxidant, and the amount of such a pro-oxidant may differ from lot to lot of the randomized oil.

Effect of purification on oxidative stability. Figure 2 shows that purification with alumina removed the postulated pro-oxidant factor from the randomized oil, because the purified natural and purified randomized oil had the same OSI. When the column polar extract, which was eluted from the alumina with methanol after the elution of the oil with the nonpolar solvent, was added back to the randomized and then purified oil, the OSI time of the oil was restored to its prepurification value. This methanol extract was further separated by TLC on a silica gel plate to identify the pro-oxidant. Various TLC bands were collected, extracted, and added back to the unpurified corn oil. Their effect on oil stability is shown in Table 5. It is obvious that MAG and DAG bands both had pro-oxidant activity, and the MAG band was more effective in reducing the OSI time of the oil. This agrees with the report of Mistry and Min (18) that MAG and DAG act as pro-oxidants at concentrations of 0.25–0.5% at 55°C in purified soybean oil and of Chung *et al*.

TABLE 2
FA Composition (%) of Natural Corn Oil (TAG) and at the *sn*-2 Position of the Natural and Randomized Corn Oils

	16:0	18:0	18:1	18:2	18:3
TAG	10.5	2.1	30.8	55.3	1.3
sn-2 before randomization	1.7	0.3	28.7	67.7	1.4
sn-2 after randomization	11.7	2.2	29.7	55.2	1.3

TABLE 4
Tocopherol Concentration (ppm) Before and After Randomization of a Corn Oil

	α	γ	δ	Total
Natural oil	222	658	33	913
Randomized corn oil	170	620	34	824

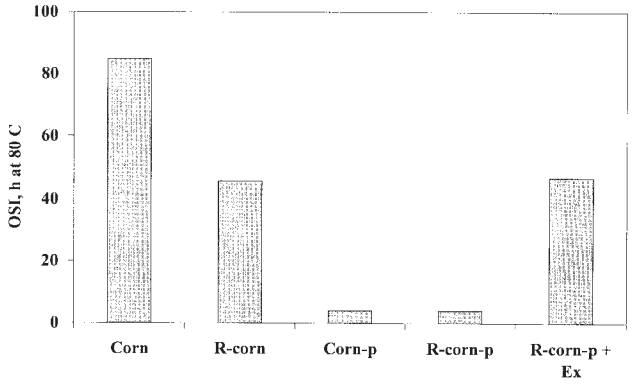

FIG. 2. Effect of alumina purification on oil oxidation. Corn, natural corn oil; R, randomized; p, purified; Ex, column polar extract by methanol. The least significant difference (at $P = 0.05$) for mean comparison is 2.5.

(19) that a 1% MAG concentration has a pro-oxidant effect in unpurified soybean oil at 55°C.

To determine whether these partial glycerides were responsible for the observed pro-oxidant effect, we quantified the MAG and DAG in natural and randomized oils by HPLC. The analysis showed that no detectable MAG was present in the natural corn oil, and that DAG was present at 1.4% by weight. In randomized oil, 0.3% MAG was produced, and DAG was increased to 5.1%. No MAG or DAG was detected in the randomized oils after alumina purification. Commercially produced, pure 18:2 MAG and DAG were added to purified corn oil, which was then oxidized at 28°C to quantify their pro-oxidant effect. These results are presented in Figure 3. Increases in oxidation rate of the purified corn oil were observed when the MAG level was 0.25% and the DAG level was 5%, but these increases were not as great as that found in the randomized oil. To determine whether the slight increase of oxidation rate caused by MAG and DAG addition in the purified oil could account for the greatly increased oxidation of unpurified randomized corn oil in which a considerable amount of tocopherols (about 824 ppm) was present, various amounts of 18:2 MAG were added to natural corn oil and oxidation was performed at 100°C by OSI. No increase in oxidation rate was observed by adding MAG up to 0.5% (OSI values of 20.3, 20.7, 20.7, and 20.6 h for the control, and 0.1, 0.25, and 0.5% MAG, respectively). An OSI of 5.9 h was observed for the unpurified, randomized oil of the same source. These results indicate that MAG cannot be the pro-oxidant responsible for the increased oxidation of the randomized corn oil.

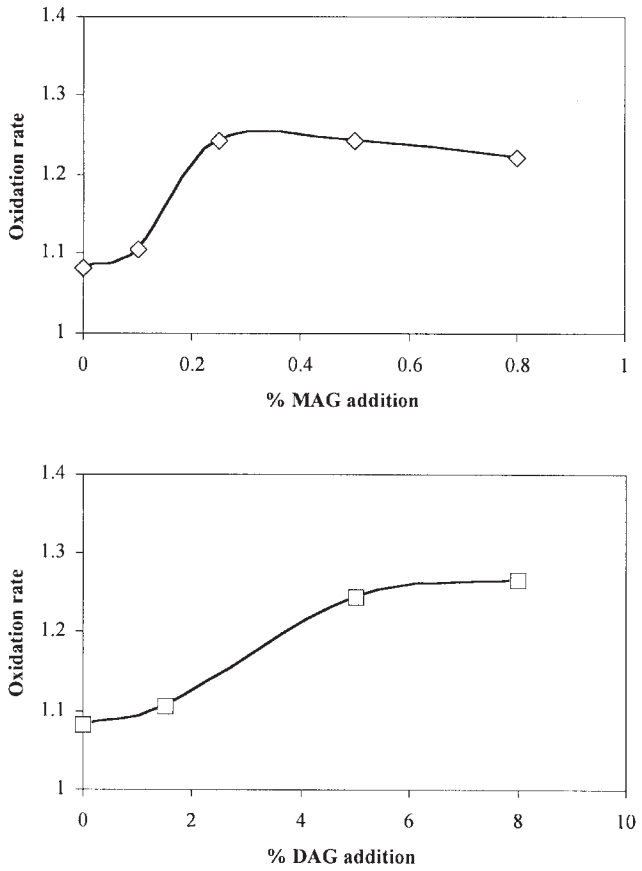

FIG. 3. Effect of MAG and DAG on the oxidation rate of an alumina-purified corn oil at 28°C.

TABLE 5
Effect of Different TLC Bands of the Polar Extract from an Alumina Column on OSI Stability of Natural Corn Oil at 100°C

TLC band	Mean OSI (h)	
	Experiment 1	Experiment 2
Untreated oil as control	18.6	20.8
Randomized oil as control	10.7	11.0
Untreated oil + total extract	10.3	14.0
Untreated oil + MAG band	12.0	8.2
Untreated oil + DAG band	14.2	13.3
Untreated oil + TAG band	19.9	ND[a]
Untreated oil + sample origin	18.3	ND
Untreated oil + silica extract	19.9	ND

[a]ND, not determined; for other abbreviation see Table 3.

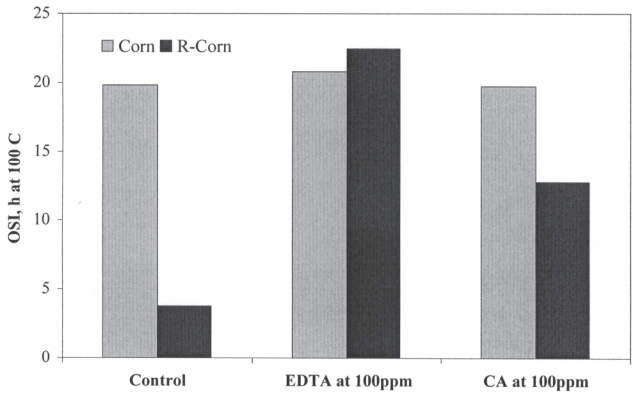

FIG. 4. Effect of chelating agent on the oxidative stability of natural and randomized corn oil. CA, citric acid; Corn, natural corn oil; R-Corn, randomized corn oil.

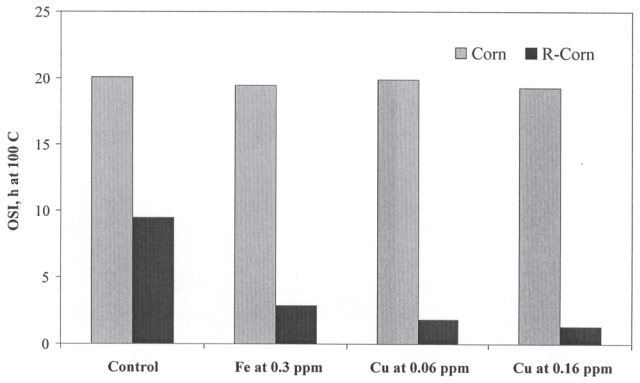

FIG. 5. Effect of adding transition metal ions on the oxidative stability (h, at 100°C) of natural and randomized oils. For abbreviations see Figure 4.

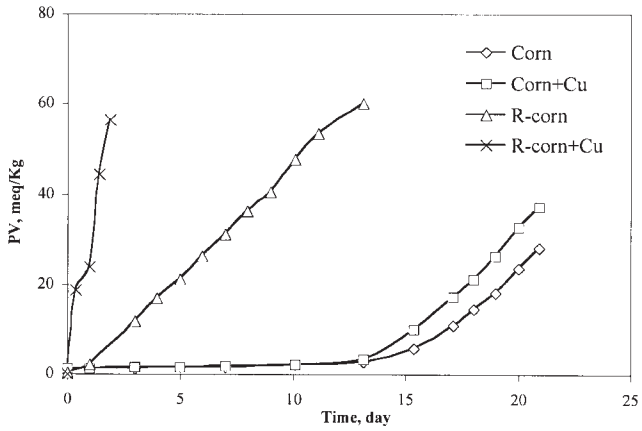

FIG. 6. Effect of cupric ion (at 0.15 ppm) on oxidation (at 50°C) of natural and randomized oils. For abbreviations see Figure 4.

There might be other compounds in the TLC bands containing MAG and DAG since extracts of these bands had shown significant pro-oxidant activity (Table 5). GC and GC–MS were used to characterize the MAG band. Significant amounts of other compounds were not found. Seemingly, if the MAG band contained other pro-oxidant compounds, they were either unstable or present in traces.

Effect of chelating agents on oxidative stability of the randomized oil. Surprisingly, EDTA and citric acid completely or partially restored the oxidative stability of the randomized oil. As shown in Figure 4, addition of 100 ppm EDTA to the randomized oil restored the OSI time to that of natural corn oil, but this amount of EDTA did not affect the OSI of the natural oil. Citric acid also fully restored the OSI time of the randomized oil at the higher concentration of 200 ppm. The effects of EDTA and citric acid on OSI were proportional to their concentration in the oil (data not shown).

These results suggest that the effect of the pro-oxidant produced during randomization depends on the presence of free metal ions in the oil, and the presence of the pro-oxidant greatly amplifies the catalytic effect of the metal ions in the oil. Metal ions are present in very low concentration in fully refined vegetable oils, e.g., 0.1–0.3 ppm of iron and 0.02–0.06 ppm of copper (20). To confirm this hypothesis, ferric and cupric ions (in the form of ferric ammonium sulfate and cupric acetate) were added to the natural and randomized corn oil, and their OSI values (h, at 100°C) were determined and are shown in Figure 5. Clearly, the addition of small amounts of ferric or cupric ions significantly decreased the OSI time of randomized corn oil, but they did not affect the stability of natural oil at the concentrations used. Therefore, the hypothesis that the pro-oxidant produced during randomization is a metal ion sensitizer seems to be true.

The effect of cupric ion on the oxidation of natural and randomized oil also was confirmed at 50°C by a PV test (Fig. 6). With cupric ion added (0.15 ppm), the randomized corn oil oxidized much faster than the control, but the oxidation of natural corn oil was not significantly affected by the addition of the cupric ion.

When cupric ion was added to the alumina-purified, randomized corn oil, the oxidation rate was not changed. The oxidation rate (at 28°C) of the randomized and then purified oil and the same oil with 0.06 ppm of cupric ion added had oxidation rates of 1.24 ± 0.07 and 1.21 ± 0.01, respectively. This result again indicates that alumina column purification effectively removed the pro-oxidant in the randomized corn oil.

To verify that trace amounts of ferric and cupric ions in the natural oil can be "sensitized" by the unknown pro-oxidant, causing the greatly increased catalytic activity, we first purified the oil by passage through an alumina column to remove the ions and then randomized the oil. This purified and then randomized oil was expected to have the same stability as the purified natural oil, because the possible presence of the pro-oxidant without ions will not affect oxidative stability. However, such oil was actually much more stable than the purified natural oil. We hypothesized that tocopherol esters present in the natural oil could have been eluted with the TAG and then converted to free tocopherol by randomization, thus contributing to the much higher stability of the purified and then randomized oil. HPLC analysis showed the presence of 6.6 ppm of γ-tocopherol in the purified and randomized oil, and adding 6.6 ppm γ-tocopherol to the alumina-purified oil indeed delayed its oxidation to the extent observed in the purified and randomized oil. This experiment showed that when the ions or the precursor of the unknown pro-oxidant was removed before randomization by alumina treatment, the pro-oxidant was either not produced or else there were no metal ions on which to exert its activity.

To understand whether the alumina purification removed the pro-oxidant precursor or metal ion, or when the pro-oxidant was generated, a corn oil was purified by the alumina column and then randomized. To this purified and then randomized oil, copper ion at a concentration of 0.06 ppm was added. The oxidation rate (slope of the natural log of PV vs. hours of oxidation) at 40°C of the oil with copper added was 0.886 ± 0.007 vs. 0.616 ± 0.012 for the oil without copper ion

addition, and the induction period of the sample with added copper was about half of that without copper ion addition. This reduction in stability may account for the much shorter OSI times of the randomized corn oil compared with the natural oil control. These data show that the pro-oxidant is generated during the randomization of the pure TAG molecules.

Liu (21) has suggested a mechanism of interesterification and proposed several intermediates, such as β-keto ester, that might be produced in this reaction. To study whether models of these intermediates had an effect on the rate of oxidation of natural corn oil, ethyl acetoacetate and ethyl diacetoacetate were tested. No significant pro-oxidant effect was found with up to 500 ppm ethyl acetoacetate in natural corn oil at 100°C in the OSI test. A small pro-oxidant effect of ethyl diacetoacetate was observed at very high level (500–5000 ppm). We also tested acetylacetone, which is known to complex with many metal ions, but observed no pro-oxidant effect. When 0.16 ppm copper was added, the OSI time of corn oil treated with any of these three substances did not change significantly.

Ascorbic acid at low concentrations (10–20 ppm) is known to form a powerful pro-oxidant with metal ions (22). Esterification on the 2-position is said to destroy its antioxidant activity (23), and esterification could allow it to elute with pure corn oil TAG during alumina purification. Ascorbate esters may be deacylated by sodium methoxide treatment, so we added ascorbic acid (0.2–9 ppm) and ascorbyl 6-palmitate (0.4–21 ppm) to natural corn oil to examine their effect on oxidative stability in the presence of naturally occurring trace metal ions. However, no pro-oxidant activity was observed in the OSI test under the same condition in which instability of the randomized oil was seen.

FFA may increase the oxidation rate because of the catalytic effect of its carboxyl group on the decomposition of hydroperoxide (24,25). The FFA level in the randomized corn oil without purification was 0.59%, compared with 0.057% in natural corn oil. To test the effect of FFA on oil oxidation, 0.6% of palmitic acid was added to corn oil, and the OSI test was conducted at 100°C. This addition did not increase the oxidation rate in corn oil.

We are uncertain why the results reported by Lau *et al*. (2) could not be repeated. Seemingly, they also produced a similar pro-oxidant or pro-oxidant by randomization of corn oil but were not able to remove it by their alumina treatment. They used more solvent to elute TAG from the alumina column than we did, and the pro-oxidant may have been eluted with the TAG.

This is the first study to report pro-oxidant formation during randomization or interesterification. Precautions or an additional purification treatment should be used in the food industry when using interesterification.

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