Effect of Randomization on Oxidative Stability of Vegetable Oils at Two Different Temperatures

C.L. Tautorus and **A.R. McCurdy***
Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO

Five vegetable oils were each randomized by chemical means (with the use of a sodium potassium alloy) and by enzymatic means (using a nonspecific lipase). The success of the randomization procedure was confirmed via positional analysis. The oxidative stabilities of the native and chemically randomized oils were determined at storage temperatures of 28°C and 55°C using absorbance at 234 nm as indicative of conjugated diene content. No difference between curves occurred in oils stored at 55°C, however, at the lower temperature all chemically randomized oils had significantly steeper slopes ($P<0.05$), suggesting a lower stability. When both enzymatically and chemically randomized oils were compared to native oils at 28°C, no significant difference occurred between slopes of native and enzymatically randomized oils, however, the end content of conjugated dienes was significantly higher for chemically randomized canola, corn and soybean oils ($P<0.05$). No difference was seen between the slopes of the three different oils from either linseed or sunflower. Since both of these oils exhibited higher oxidation rates, it is possible that observation of differences between the stability of native and chemically randomized oils is dependent upon the rate of the reaction.

KEY WORDS: Canola oil, corn oil, linseed oil, oxidative stability, randomization, soybean oil, sunflower oil.

There have been numerous reports published on the effect of chemical randomization on the oxidative stability of triacylglycerols (1-6). Chemically randomized oils have been described as expressing either an increased or an equivalent rate of autoxidation, in comparison with the original native oil. Lau and Hammond (3) found a three-four-fold increase in the rate of autoxidation of randomized, over that of native corn oil, while Park *et al.* (4) found no difference in autoxidative rate between randomized and native soybean triacylglycerols.

A survey of the literature shows that published studies have varied in experimental conditions, utilizing different oils and storage temperatures. For example, while Lau and Hammond (3) used corn oil stored at 28°C, Park *et al.* (4) studied soybean oil stored at 60°C. It is possible that the autoxidation temperature and/or the composition of the oil used may have had an effect on the observation of any differences noted in autoxidation rate. Accordingly, in the first part of this study, both a comparatively high (55°C) and a low (28°C) storage temperature were employed. In addition, in order to determine whether the composition of the oil had any effect on the extent of any observed differences in autoxidation rate between native and randomized oils, five different vegetable oils were employed.

An additional consideration in a study of the effect of randomization on autoxidative stability is the possibility that the method of randomization itself may be responsible for any observed difference noted in autoxidation rate between native and randomized oils. Recently, it has become possible to readily randomize triacylglycerols by enzymatic means. To reveal any effect on oxidative stability caused solely by the chemical randomization, in the second part of the study reported here, the vegetable oils used were also randomized by enzymatic means, using an immobilized non-specific lipase manufactured by Novo(SP-382). The oxidative stability of such oils were then compared to native and chemically randomized oils.

EXPERIMENTAL PROCEDURES

Canola, corn, soybean and sunflower oils were obtained from commercial sources. Linseed oil was obtained by cold pressing seed through a Rosedowns Mini 40 Screw Press (Simon Rosedowns, Ltd., Hull, England), followed by centrifugation of expelled oil at 5,000 × *g* for 25 min to remove the fines. Each oil was purified with alumina (7), and subsequent purity was confirmed as only containing triacylglycerols via thin-layer chromatography with hexane:diethyl ether:acetic acid (50:50:0.5) as developing solvent and iodine vapor for visualization. Purification was necessary to ensure that all oils were free of antioxidants and any contaminants that could have affected either the randomization reactions or the stability tests.

Chemical randomization was performed by continuously stirring a mixture of dry oil and 0.2% (w/w) sodium potassium alloy for 2 hr at 80°C under nitrogen (8). After three washes of the reaction mixture with equal volumes of distilled water, the subsequent products were dried by heating to 150°C, then purified by passage through an alumina column. Purity was confirmed via thin-layer chromatography.

Enzymatic randomization was performed by continuously stirring a mixture of oil and 5% (w/w) immobilized non-specific lipase (SP - 382, Novo Nordisk Bioindustrials, Inc., Danbury, CT) for 24 hr at 60°C under nitrogen. The enzyme contained 10% moisture on an as is basis. All oils were purified after enzymatic randomization via an alumina column, then subjected to thin-layer chromatography to ascertain purity.

Successful randomization of oils was confirmed by positional analysis through the method of Pan and Hammond (9). In each analysis, the validity of the results was ascertained by determining the 1,3 specificity, fatty acid non-specificity of each digestion with pancreatic lipase through comparison of the expected with the obtained 1,2 and 2,3 diacylglycerols. Fatty

*To whom correspondence should be addressed.

acid analysis was performed on a Hewlett Packard Model 5700A gas chromatograph (Packard Instruments, Downers Grove, IL) equipped with a flame ionization detector, utilizing a 30m WB SupelcoWax 10 capillary column (I.D. 0.32mm) (Supelco, Inc., Bellefonte, PA) at an isothermal temperature of 220°C. The carrier gas was hydrogen at 1 mL/min flow rate.

Oxidations were carried out without stirring on 0.5 g quantities of oil in 15 × 45 mm vials in the dark at 28°C or 55°C. Extent of oxidation was assessed every 24 hr or every 12 hr for oils stored at 28°C or 55°C, respectively. This was done by determining the absorbance of aliquots of oil in hexane at 234 nm. Values so obtained are representative of the conjugated diene content (10). Data points are the result of averaged duplicate samples.

Statistics were performed on the straight line portion of the curves for each oil, using Student's t-test or analyses of covariance when comparing sets of two or three lines, respectively. All tests of significance were carried out at a 0.05 level of probability. If the analyses of covariance of three lines revealed the obtained F statistic to be significant at this level, a Newman-Keul's multiple range test was employed to determine which lines differed significantly.

RESULTS

Table 1 presents the results of positional analyses of the oils used in the first part of this study before and after chemical randomization. No great change was seen in the triacylglycerol fatty acid composition of any oil as a result of randomization. However, in the randomized oils, the content of fatty acids located at the *sn*-2 position was seen to approach that of the whole triacylglycerol, indicating that the chemical method was successful in randomizing fatty acid distribution.

Figures 1a–1d and 2 indicate the extent of oxidation with time as measured by conjugated diene content of native and chemically randomized oils at two different temperatures. In every case, the rate of increase of these oxidation products as measured in both the native and the randomized oil was higher when stored at 55°C than at 28°C. In addition, a trend can be seen with the more highly unsaturated oils expressing a higher rate of accumulation of conjugated dienes, with the highly unsaturated linseed and the comparatively less unsaturated corn and canola oils being the extremes.

A secondary effect of temperature is seen when the stability of randomized and native oils are compared within each temperature. Such a comparison shows the expression of a difference in stability to be temperature dependent. At 55°C, no significant difference was apparent between the slopes of the straight line portions of the curves representing the rate of accumulation of oxidation products of the native and chemically randomized corn, linseed, sunflower and soybean oils. No statistical analysis was performed on the curves representing canola oil stored at 55°C, as no straight line portion with sufficient data points was observed. A difference in slope of the resulting curves for all native and randomized oils did occur, however, when the oils were stored at 28°C. Statistical analyses of these lines indicated the differences in slopes between the native and randomized oils to be significant in every case, and with the exception of linseed, the chemically randomized oils were less stable.

TABLE 1

Fatty Acid (Mole Percent) Distribution Within Whole Triacylglycerol (TAG) and at *sn*-2 Position Monoacylglycerol (*sn*-2 MAG) in Native and Chemically Randomized Vegetable Oils

Vegetable oil	16:0	18:0	18:1	18:2	18:3
Canola oil native TAG	4.7	1.7	68.5	19.3	5.8
sn-2 MAG	1.0	0.0	62.0	28.5	8.3
chemically randomized TAG	4.9	1.6	71.3	18.0	4.2
sn-2 MAG	5.4	1.7	69.1	18.6	5.1
Corn oil native TAG	10.3	1.7	30.4	57.1	0.5
sn-2- MAG	2.3	1.1	27.6	68.5	0.6
chemically randomized TAG	10.5	1.4	28.2	59.9	0.0
sn-2 MAG	11.3	1.1	28.1	59.0	0.0
re-randomized TAG	11.1	1.3	28.4	59.3	0.0
sn-2 MAG	13.5	1.1	29.4	54.9	0.0
Linseed oil native TAG	6.1	3.2	12.9	13.2	64.6
sn-2 MAG	1.3	0.5	15.0	21.1	62.1
chemically randomized TAG	6.3	3.4	13.3	13.7	63.3
sn-2 MAG	6.4	3.4	12.5	12.9	64.7
Soybean oil native TAG	11.5	2.9	21.3	56.6	6.6
sn-2 MAG	2.0	0.0	20.2	70.9	6.4
chemically randomized TAG	11.4	3.1	22.0	57.5	5.8
sn-2 MAG	12.0	3.4	21.5	55.8	7.3
Sunflower oil native TAG	7.3	5.2	16.4	71.7	0.0
sn-2 MAG	1.2	1.0	14.0	83.8	0.0
chemically randomized TAG	7.2	5.5	16.4	71.0	0.0
sn-2 MAG	8.7	7.6	17.5	66.2	0.0

Figure 2 also shows the result of storage at 55°C and at 28°C of randomized corn oil that had been subjected to an additional randomization treatment. In both cases, no further significant change occurred in the stability of the re-randomized oil, in comparison to the once randomized oil.

Table 2 shows the results of a positional analysis of the oils used in the second part of this study before and after enzymatic and chemical randomization. Again, no great change was seen in the triacylglycerol fatty acid composition of any oil as a result of randomization by either method. A comparison of the content of fatty acids located at the *sn*-2 position in the randomized and native oils reveals randomization to have been successful by both chemical and enzymatic means.

Figures 3a–e show the results of storage at 28°C of the native, chemically and enzymatically randomized oils. In the case of canola and corn oils, no large difference occurred in the slopes of curves representing accumulation of conjugated dienes between the native and enzymatically randomized oils, whereas the curves representing chemically randomized oils exhibited steeper slopes, suggesting a faster rate of oxidation. Statistical analysis showed that while the rate of accumulation of conjugated dienes was significantly greater in the chemically interesterified oils, there was no significant difference detected between the rates of the native and the enzymatically interesterified oils.

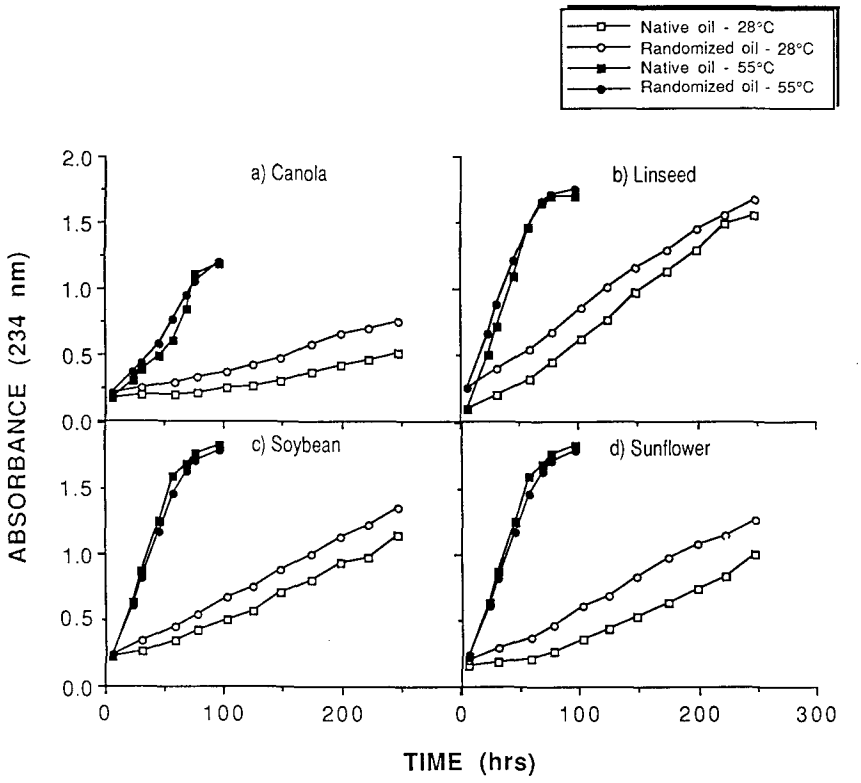

FIG. 1. Lipid oxidation, as measured by absorbance at 235 nm (indicative of conjugated diene content) of various native and chemically randomized vegetable oils.

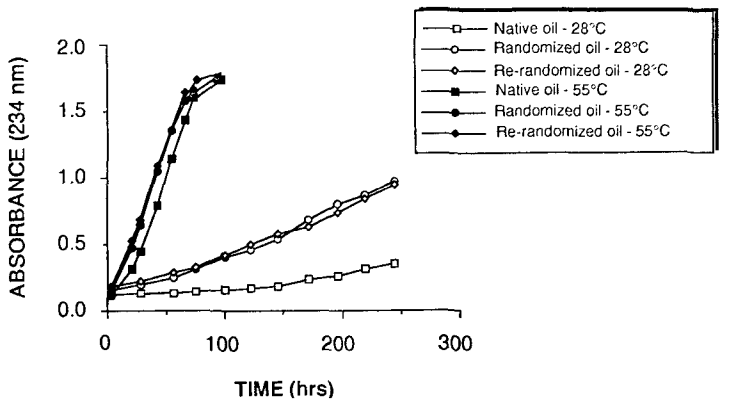

FIG. 2. Lipid oxidation, as measured by absorbance at 234 nm, with time for native, chemically randomized and re-randomized corn oils.

In the case of linseed oil, no significant differences were noted between the slopes of any of the three lines. Similarly, no difference was detected between the slopes of the three lines representing the soybean oils, although the apparent presence of a slight induction period for the native and enzymatically randomized oils resulted in a lower final accumulation of conjugated dienes. The results obtained for sunflower oil varied significantly from those of the other oils, with the enzymatically randomized oil exhibiting an initially higher content of conjugated dienes than either the chemically randomized or native oils. It is unknown why this should occur, but was observed again when the experiment was repeated. Statistical analysis of the slopes of the initial straight line portion of each of the curves representing the three sunflower based oils

TABLE 2

Fatty Acid (Mole Percent) Distribution Within Whole Triacylglycerol (TAG) and at *sn*-2 Position Monoacylglycerol (*sn*-2 MAG) in Native, Chemically and Enzymatically Randomized Vegetable Oils

Vegetable oil	16:0	18:0	18:1	18:2	18:3
Canola oil native TAG	5.3	2.4	67.0	20.4	5.0
sn-2 MAG	0.0	0.8	59.3	31.2	8.7
chemically randomized TAG	5.1	2.5	68.9	19.9	3.8
sn-2 MAG	5.1	2.7	68.1	19.3	4.8
enzymatically randomized TAG	4.8	2.4	70.0	19.2	3.7
sn-2 MAG	6.2	3.3	67.9	18.3	4.1
Corn oil native TAG	9.6	1.8	30.5	56.8	0.8
sn-2- MAG	1.2	0.4	28.0	70.4	0.0
chemically randomized TAG	9.9	2.0	32.1	55.4	0.7
sn-2 MAG	10.9	2.6	31.9	54.0	0.0
enzymatically randomized TAG	9.7	2.0	32.9	54.9	0.6
sn-2 MAG	10.2	2.4	31.9	54.7	0.7
Linseed oil native TAG	5.7	3.5	13.3	15.7	61.9
sn-2 MAG	0.7	0.3	14.2	22.3	62.5
chemically randomized TAG	5.9	3.8	14.3	16.2	59.9
sn-2 MAG	5.9	3.7	13.2	14.8	59.9
enzymatically randomized TAG	6.4	3.8	14.7	16.4	58.8
sn-2 MAG	6.0	4.0	13.7	15.5	60.7
Soybean oil native TAG	12.0	3.7	23.2	54.3	6.8
sn-2 MAG	1.0	0.0	20.3	71.6	6.9
chemically randomized TAG	11.6	4.1	24.4	53.9	6.1
sn-2 MAG	11.1	4.4	23.4	52.1	6.5
enzymatically randomized TAG	11.8	4.0	24.0	54.0	6.4
sn-2 MAG	11.9	4.2	22.9	53.7	7.1
Sunflower oil native TAG	6.6	5.4	16.1	71.9	0.0
sn-2 MAG	0.7	0.0	13.9	85.3	0.0
chemically randomized TAG	7.6	5.4	16.2	70.8	0.0
sn-2 MAG	8.3	5.3	14.9	71.3	0.0
enzymatically randomized TAG	7.4	5.4	16.7	70.6	0.0
sn-2 MAG	5.1	4.0	15.3	75.6	0.0

revealed no significant difference between the rate of accumulation of conjugated dienes.

DISCUSSION

A positional analysis was performed on the native oils and after chemical and enzymatic randomization to determine whether any notable change had occurred in fatty acid composition due to the treatments and whether randomization had actually occurred. The success of both the chemical and the enzymatic randomization procedure used was shown by the results of these analyses, as in each case the randomization method used resulted in a randomized oil. The tendency of polyunsaturated fatty acids to occupy the middle *sn*-2 position of the TAG, a characteristic of vegetable oils that has been frequently observed by previous researchers (9,11,12) is also seen in Tables 1 and 2. It is interesting to note that oleic acid follows the saturated fatty acids stearic and palmitic in being located preferentially at positions 1 and 3 of these vegetable oil TAGs.

It is apparent from a comparison of the stabilities of the chemically randomized and native oils oxidized in the first part of this study that previous conclusions (2-5) regarding the absence or presence of a protective effect provided by the native organization of the fatty acids of an oil were not due to the use of different vegetable oils. In the present study, both corn and soybean in a randomized state exhibited a higher rate of conjugated diene accumulation when stored at 28°C. However, it is clear that the different storage temperatures previously used could have exerted a strong influence on the assessment of oxidative stability. The differences apparent between chemically randomized and native oils noted at 28°C disappeared when the oils were stored at 55°C. The effect of temperature on assessment of storage stability has been noted by Warner *et al.* (13), who found the relative stabilities of low erucic acid rapeseed, soybean and sunflower oils to be dependent upon the test condition temperature.

However, it may be that in this case, observation of the protective effect in particular may depend on temperature to the extent that lower temperatures generally result in a relatively slow rate of oxidation. This is supported by the results obtained with the linseed based oils, which even at the lower storage temperature had a relatively high rate of accumulation of conjugated dienes and showed the least difference between the stability of the native and chemically randomized oils.

Examination of results obtained from the second part of the study shows the varying effects of randomization as accomplished by chemical vs enzymatic means. For both canola and corn oils, randomization through chemical means resulted in an oil that was less stable than either the enzymatically randomized or native oils. Equivalent rates were exhibited by chemically randomized and native soybean oils; unlike the results obtained from the first part of this study, however, the native and enzymatically randomized oils expressed a longer induction period, resulting in a lower end accumulation of conjugated dienes. As before, no significant difference was observed between the three curves obtained for the linseed oils. This result negates the possibility that the enzyme treatment was adding an antioxidant to the system that was passed through the column, as if this were the case, all enzymatically randomized oils should have expressed a higher stability. The sunflower oils as well did not appear to be different in their stability, unlike the observation in the first part of the study. However, the overall rates of oxidation of the sunflower based oils were higher than those noted in the first part of the study, which once again could have obscured any difference in stability as a result of method of randomization. It is unknown why the rates should have been higher, as the same oil source, as well as the same preparation methods, were used.

The results of both of these experiments support previous studies done at low storage temperatures which showed chemically randomized vegetable oils to be less oxidatively stable than the native oil. However, when randomization was accomplished by other means (i.e., enzymatic) no detectable difference was observed, suggesting the previous effect was a result of the chemical randomization reaction itself. The lack of any further effect shown by the re-randomization of the corn oil suggests that the effect of chemical randomization occurs during the initial reaction and is not further intensified by subsequent exposures. It is likely that some

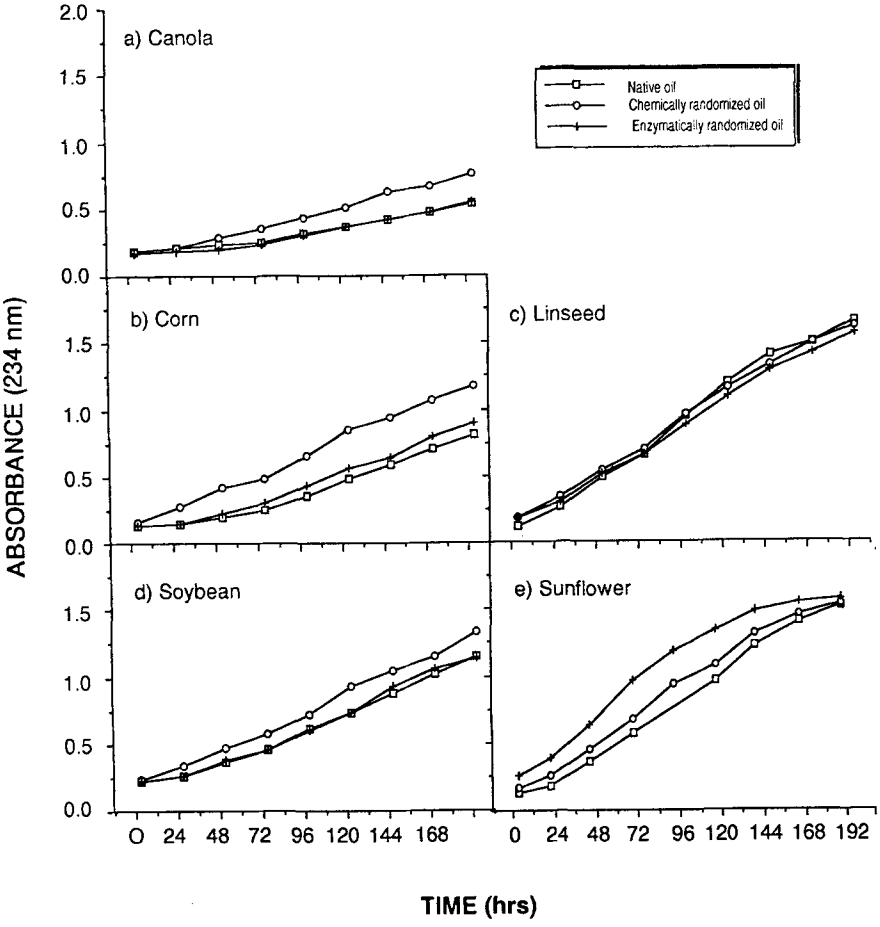

FIG. 3. Lipid oxidation, as measured by absorbance at 234 nm, with time for native, chemically and enzymatically randomized oils stored at 28°C.

factor is being removed from the oil during the initial reaction that is not removed further during the subsequent reaction. That something is being removed is more reasonable than if a factor were being added to the system, as such a result should be additive with repeated exposure to the treatment. It is also more likely that differences occurring with the removal of a factor from an oil would not be remedied by the use of a purifying column, which would be the case if something were added to the system.

It is possible that a protective effect exists that was not observed in the oils used in this study due to their low ratio of saturated to unsaturated fatty acids. In the original paper by Raghuveer and Hammond (1), it was suggested that fats high in unsaturated fatty acids would exhibit any protective effect to a decreased extent. Therefore, it is possible that none of the vegetable oils used in this study have a sufficiently high saturated to unsaturated fatty acid ratio to express a decrease in oxidative stability caused by randomization. Further research is required with oils possessing higher saturated to unsaturated fatty acid ratios in order to finally ascertain the existence of the proposed protective effect.

ACKNOWLEDGMENTS

The authors thank H. Braitenbach of the Crop Science Department, University of Saskatchewan, for processing of the linseed, G. Slater of the Plant Biotechnical Institute, National Research Council, for the use of the gas chromatograph as well as invaluable advice, and Novo Bioindustrial Group for providing the enzyme. This research was funded by the Canada/Saskatchewan ERDA agreement.

REFERENCES

1. Raghuveer, K.G., and E.G. Hammond, *J. Am. Oil Chem. Soc. 44*:239 (1967).
2. Fatemi, S.H., and E.G. Hammond, *Lipids 15*:379 (1980).
3. Lau, F.Y., and E.G. Hammond, *J. Am. Oil Chem. Soc. 459*:407 (1982).
4. Park, D.K., J. Terao and S. Matsushita, *Agric. Biol. Chem. 47*:121 (1983).
5. Park, D.K., J. Terao and S. Matsushita, *Ibid. 47*:2243 (1983).
6. Wada, S., and C. Koizumi, *J. Am. Oil Chem. Soc. 460*:1105 (1983).

7. Jensen, R.G., T.A. Marks, J. Sampugna, J.G. Quinn and D.L. Carpenter, *Lipids 1*:451 (1966).
8. Derosier, W.J., M.Sc. Thesis, University of Saskatchewan (1988).
9. Pan, W.P., and E.G. Hammond, *Lipids 18*:882 (1983).
10. Madison, B.L., and W.J. Hughes, *J. Assoc. Off. Anal. Chem. 66*:81 (1983).
11. Mattson, F.H., and E.S. Lutton, *J. Biol. Chem. 233*:868 (1958).
12. Brockerhoff, H., *J. Lipid Res. 6*:10 (1965).
13. Warner, K., E.N. Frankel and T.L. Mounts, *J. Am. Oil Chem. Soc. 466*:558 (1989).

[Received November 30, 1989; accepted April 12, 1990]