Epoxide Yield Determination of Oils and Fatty Acid Methyl Esters Using ^{1}H NMR

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ABSTRACT: Product mixtures of epoxidized fatty compounds can be analyzed by using ^{1}H NMR. Conversion of double bonds and selectivities to different products can easily be calculated. Moreover, if diunsaturated substrates are used in epoxidation reactions, yields to mono- and diepoxidized products can be determined. The effectiveness of this method is proven by comparing some NMR results with those found by GC analysis.

Paper no. J10770 in *JAOCS 81*, 841–846 (September 2004).

KEY WORDS: Epoxidized fatty acids, epoxidized oils, ^{1}H NMR.

The high environmental burden caused by the use of nonrenewable petrochemical-based feedstock for the chemical industry has led to the search for vegetable oil-based alternatives. In this perspective, as in petrochemical reactions, fatty epoxides are very important because they can be used as intermediates for the production of a variety of chemicals. Up to now, most of these reaction mixtures have been analyzed by using a combination of iodine value and oxirane oxygen content (1–3). This method, however, is labor-intensive and not suitable if many samples have to be analyzed. Although GC also can be used, this method can only be applied directly to FA or their alkyl esters. If fats or oils are used as substrates, transesterification is necessary, which makes the analysis time-consuming (4) and less accurate.

Nowadays, as NMR apparatus is becoming standard equipment in the chemical laboratory, it is realistic to think future measurements will routinely be carried out this way. The use of ^{1}H NMR to determine transesterification yields (5) and the composition of vegetable oils (6) has already been reported. Although work has been done identifying oxirane functional groups within FA chains (7), no efforts have been reported using this method for quantitative analysis.

Here, we report our findings on the use of ^{1}H NMR to quantify the yield of fatty epoxides formed in different oxidation reactions directly. A variety of substrates such as methyl oleate, methyl linoleate, high-oleic sunflower oil, and safflower oil are oxidized and analyzed using ^{1}H NMR. Different products are easily identified and quantified with little workup. To show the reliability of the method, comparison is made between GC and ^{1}H NMR and the reproducibility of the method is verified.

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EXPERIMENTAL PROCEDURES

Materials. Methyl oleate (99%+) and methyl linoleate (99%) were purchased from MP Biochemicals (Asse, Belgium) and used as received. High-oleic sunflower oil (SUN) and safflower oil (SAF) containing 90% trioleate and 80% trilinoleate, respectively, were both received from N.V. Oleon (Oelegem, Belgium). $CDCl_3$ (1% trimethylsilyl), $CHCl_3$, 3-chloroperbenzoic acid (*m*CPBA), and $MgSO_4$ (anhydrous) were purchased from Acros (Geel, Belgium).

Analysis using ^{1}H NMR. Small amounts of substrate and product mixtures were dissolved in 0.5 mL of $CDCl_3$. ^{1}H NMR spectra were recorded on a 300 MHz Bruker Avance NMR spectrometer with a magnetic field of 7.05 T. Only eight scans were needed to obtain a clear spectrum.

Analysis using GC. Small amounts of epoxidized alkyl esters were dissolved in $CHCl_3$. GC was performed on a Hewlett-Packard model 6890 gas chromatograph (N_2 carrier gas) equipped with an FID. A polar BPX 70 column (0.32 mm i.d. × 60 m) from SGE (Melbourne, Australia) was used with the following temperature program: 180°C (0 min), 2.5°C/min to 240°C, 240°C (20 min).

Epoxidation using m*CPBA*. Fatty compound (2 mmol) is dissolved in 5 or 10 mL of $CHCl_3$. Stoichiometric amounts of *m*CPBA are added. The reaction mixture is stirred at room temperature for about 20 min, after which water is added to remove the *m*CPBA. The organic layer is separated, dried with $MgSO_4$, and evaporated under reduced pressure.

RESULTS AND DISCUSSION

Epoxides of methyl oleate, SUN, methyl linoleate, and SAF are prepared using *m*CPBA as described in the Experimental Procedures section. They are all analyzed using ^{1}H NMR; spectra of the different substrates and products are given in Figures 1 to 4. It is important to see the analogy between the spectra of the vegetable oils and their methyl esters; differences are due to the ester function. The assignments of the chemical shifts of important protons for glycerides and methyl esters (substrates and products) are summarized in Schemes 1 and 2.

Reaction yields can be determined by evaluating the integration values of both the appearing and disappearing signals. To illustrate this feature, oxidation of methyl oleate with *m*CPBA is monitored over time by ^{1}H NMR, as shown in Figure 5. One can clearly see a decrease of the peak area at 2.01

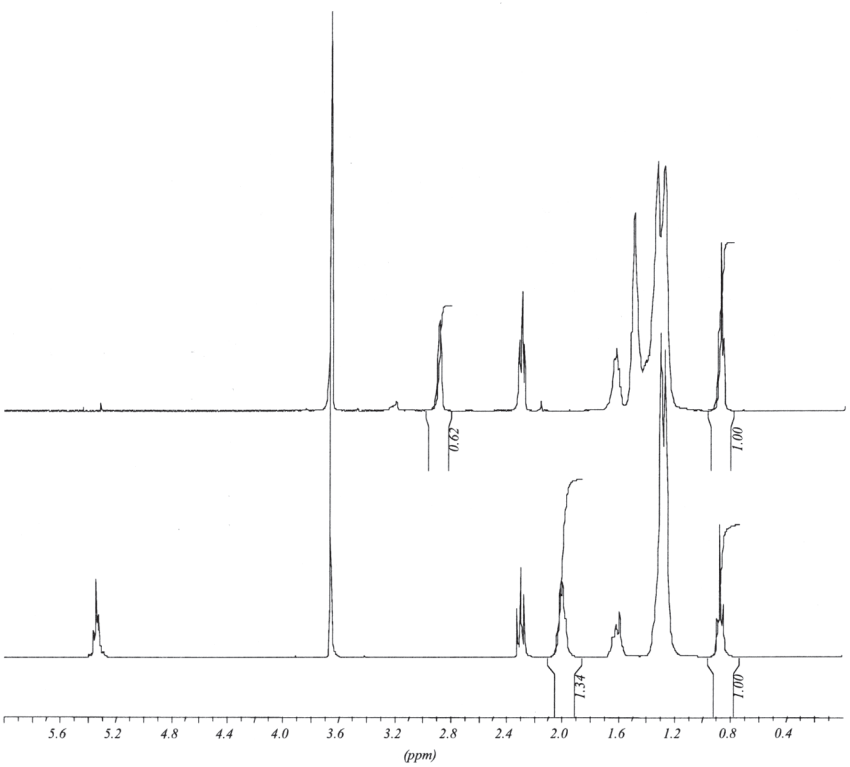


FIG. 1. [1]H NMR spectrum of methyl oleate (lower) and epoxidized methyl oleate (upper).

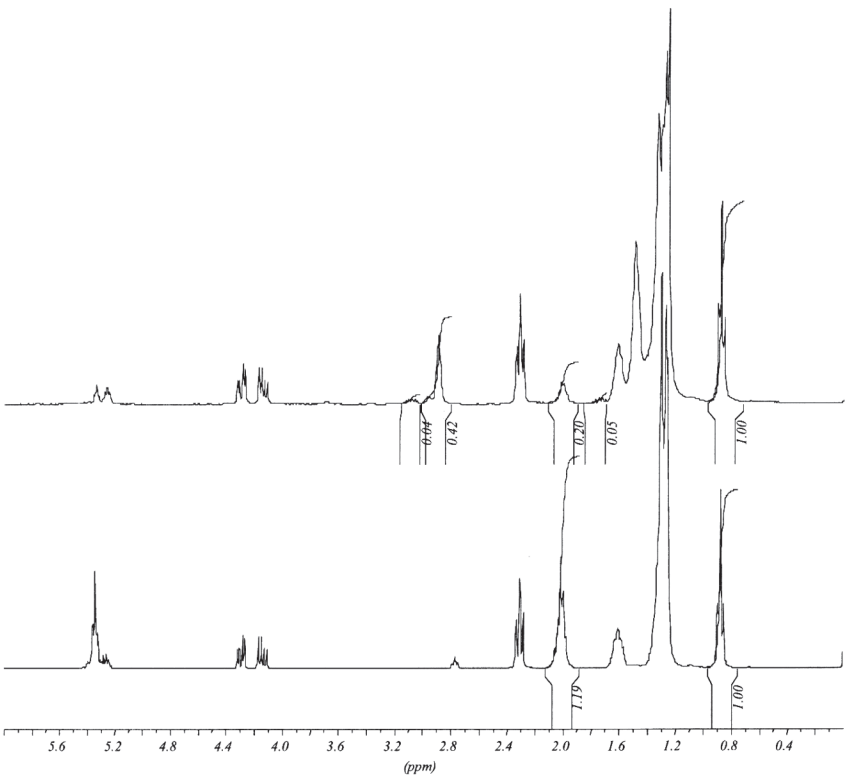


FIG. 2. [1]H NMR spectrum of high-oleic sunflower oil (SUN) (lower) and epoxidized SUN (upper).

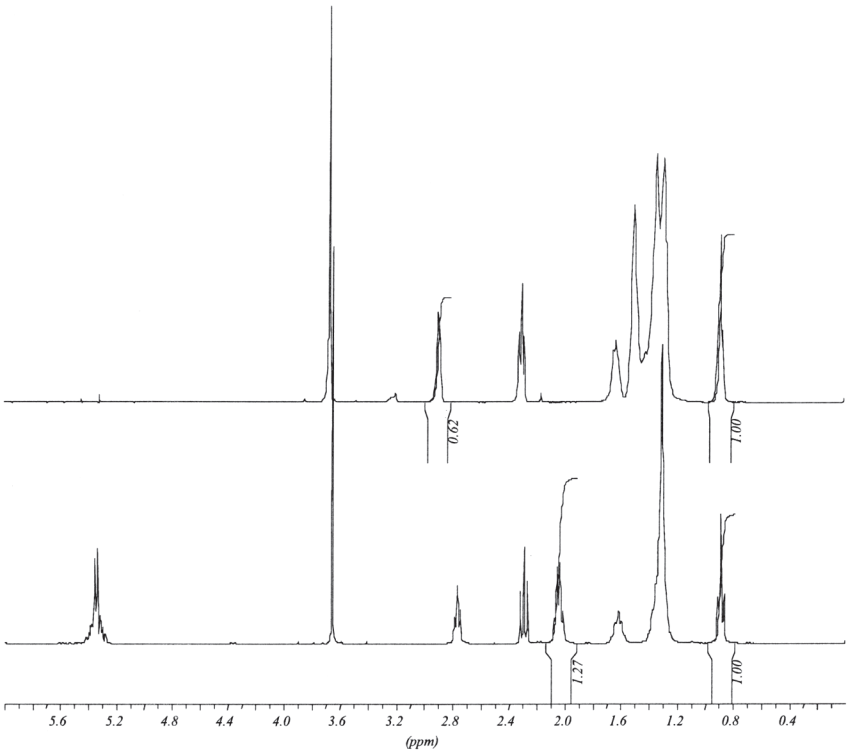


FIG. 3. ^{1}H NMR spectrum of methyl linoleate (lower) and epoxidized methyl linoleate (upper).

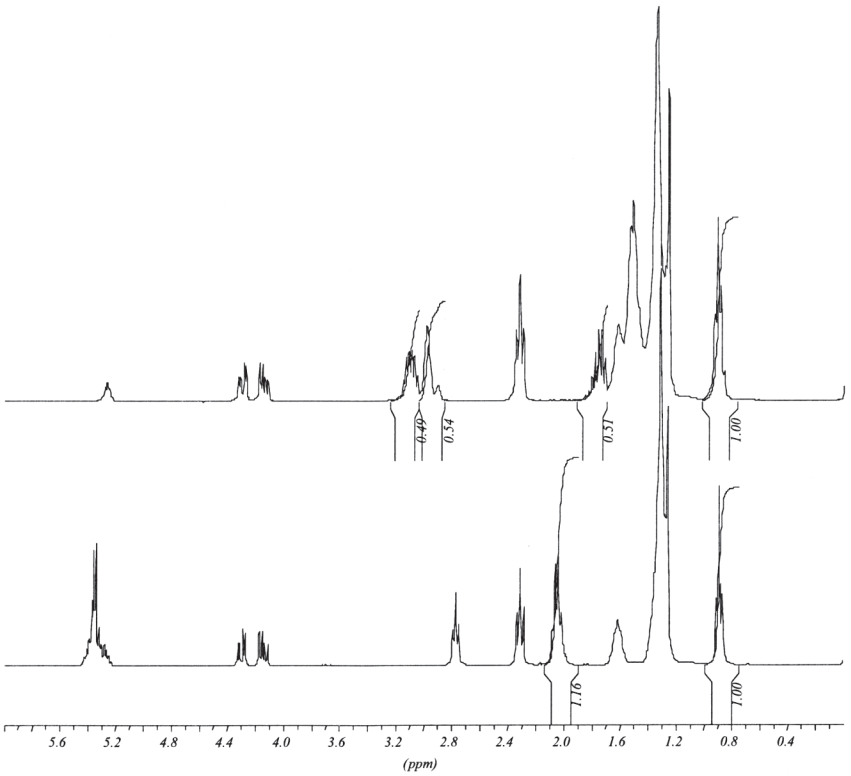


FIG. 4. ^{1}H NMR spectrum of safflower oil (SAF) (lower) and epoxidized SAF (upper).

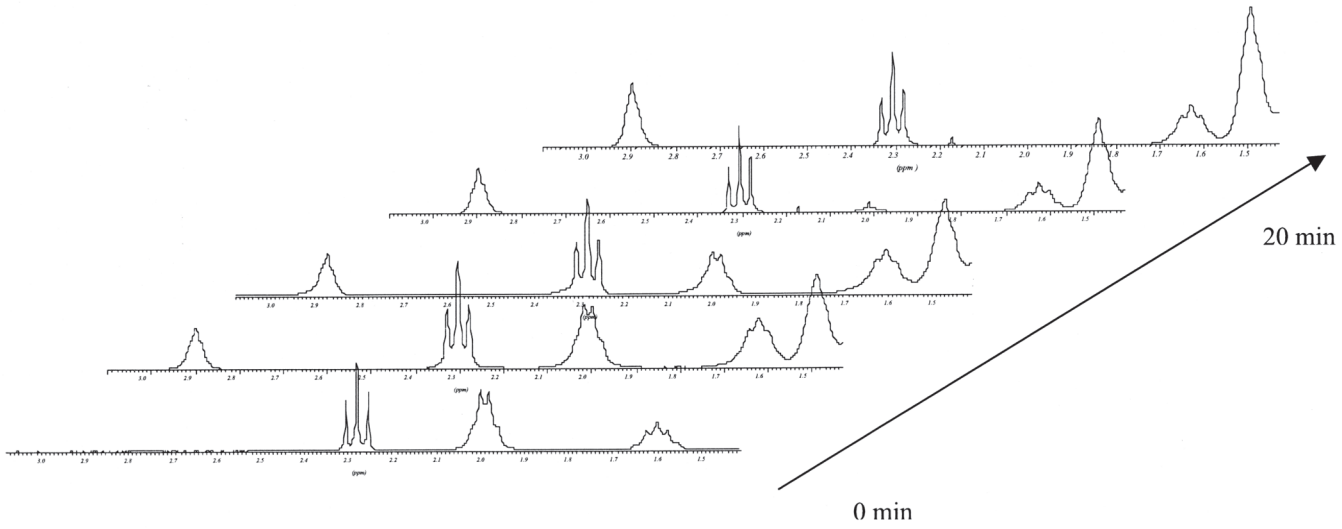


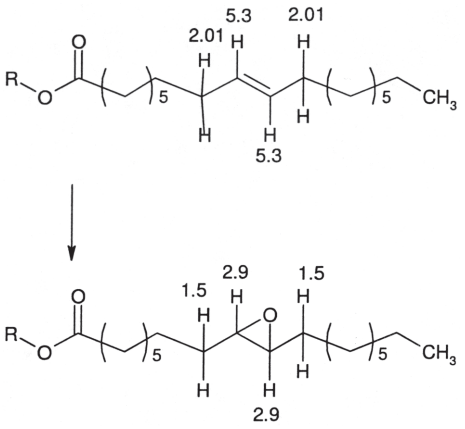
FIG. 5. Oxidation of methyl oleate over time monitored by ^{1}H NMR.

ppm, from the –C**H**$_2$–CH=CH–C**H**$_2$– protons, whereas new signals are arising at 1.50 (–C**H**$_2$–CHOCH–C**H**$_2$–) and 2.90 ppm (–C**H**OC**H**–).

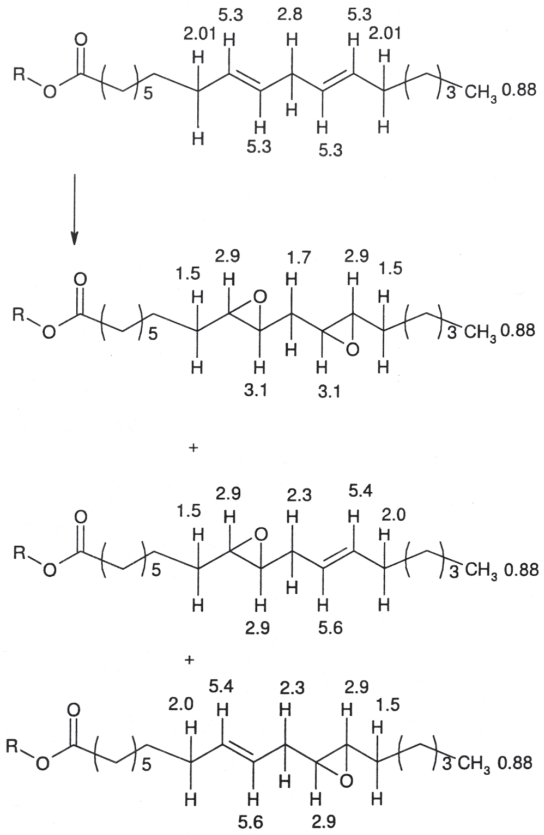
Only the most relevant signals at 2.01 (*m*) and 2.90 (*m*) ppm for oleic and at 2.01 (–C**H**$_2$–CH=CH–CH$_2$–CH=CH–C**H**$_2$–, *m*), 2.90 (*m*), and 3.10 (*m*) ppm (both oxirane protons) for linoleic species are used for integration, with both using the signal at 0.88 ppm (–C**H**$_3$, *t*) as internal standard because it is unaltered during reaction. The signal at 2.3 ppm (–C**H**$_2$–COOR) also can be used for purposes of an internal standard; the same results are obtained.

As for determination of conversion, the same procedure is applied for oleic- and linoleic-based species. Reaction conversions, denoted as *X*, of the double bond(s), at time *t*, can be determined by using the peak intensity at 2.01 ppm ($A_{2,t}$) as no interference from other signals occurs. Because the peak area at 0.88 ppm ($A_{S,t}$) remains unaffected by the reaction, it can be taken as the internal standard. Equation 1 has been used to determine the reaction conversion of double bonds of the methyl esters as well as of fats and oils:

$$X\,(\%) = 100 \cdot \left[\frac{(N_S \cdot A_{2,0} / N_{2.01} \cdot A_{S,0}) - (N_S \cdot A_{2,t} / N_{2.01} \cdot A_{S,t})}{N_S \cdot A_{2,0} / N_{2.01} \cdot A_{S,0}} \right] \quad [1]$$



SCHEME 1



SCHEME 2

TABLE 1
Analysis of Epoxidation Reaction Mixtures[a] Using ^{1}H NMR and GC

Substrate	Oxidant	Amount oxidant (mmol)	^{1}H NMR			GC		
			S_{MO} (%)	S_{DO} (%)	X (%)	S_{MO} (%)	S_{DO} (%)	X (%)
Methyl oleate	*m*CPBA	1	96	—	42	100	—	46
Methyl oleate	*m*CPBA	2	100	—	99	100	—	100
Methyl linoleate	*m*CPBA	1	72	28	53	84	16	51
Methyl linoleate	*m*CPBA	2	0	95	100	0	93	99

[a]Conditions: 1 (or 2 mmol) substrate + stoichiometric amount of oxidant 3-chloroperbenzoic acid (*m*CPBA), in 5 mL of $CHCl_3$, were reacted at 293 K for 20 min. S_{MO}, selectivity to monoepoxide; S_{DO}, selectivity to diepoxide; X, conversion of double bonds.

where $A_{2,0}$ and $A_{2,t}$ are the intensities of the signals at 2.01 ppm in the substrate and in the product spectrum and $A_{S,0}$ and $A_{S,t}$ are the intensities of the resonance peaks of the internal standard in the substrate and the product spectrum. N_S represents the amount of protons of the internal standard, whereas $N_{2,01}$ stands for the amount of protons of the signal at 2.01 ppm ($N_{2.01} = 4$).

With respect to selectivity, only one epoxide product can be formed from oleic species, whereas for linoleic species mono- and diepoxides are formed. Reaction selectivities S for oleic species at time t can be calculated by using the signal intensities of the peaks at 2.9 ppm (–C**H**OC**H**–) and other possible peaks from by-products taking into account the amount of hydrogen atoms. Equation 2 can be used to determine selectivities for the epoxide:

$$S_{\text{Epox}}(\%) = 100 \cdot \left[\frac{1 \cdot A_{2.9,t} / N_{2.9} \cdot A_{S,t}}{A_{2.9,t} / N_{2.9} \cdot A_{S,t}) + \sum_z A_{z,t} / N_z \cdot A_{S,t}} \right] \quad [2]$$

where $A_{2.9,t}$ and $A_{S,t}$ are the intensities of the peaks at 2.9 and 0.88 ppm (internal standard) and $A_{z,t}$ are the intensities of the by-products peaks. $N_{2.9}$ and N_z are the amounts of protons for which a peak stands (e.g., $N_{2.9} = 2$). Since we observed none during our reaction, the by-products term drops out in Equation 2.

Reaction selectivities for linoleic species are calculated from signals at 2.9 and 3.1 ppm. Because the signal intensity at 2.9 ppm ($A_{2.9,t}$) is due to the protons of the mono- and diepoxide and the intensity of the peak at 3.1 ppm ($A_{3.1,t}$) only to those of the diepoxide, selectivities to these partially and fully oxidized species can be calculated using Equations 3 and 4:

$$S_{\text{MO}}(\%) = 100 \cdot \left[\frac{(1 \cdot A_{2.9,t} / N_{2.9} \cdot A_{S,t}) - (1 \cdot A_{3.1,t} / N_{3.1} \cdot A_{S,t})}{(1 \cdot A_{2.9,t} / N_{2.9} \cdot A_{S,t}) + \sum_z (1 \cdot A_{z,t} / N_z \cdot A_{S,t})} \right] \quad [3]$$

$$S_{\text{DO}}(\%) = 100 - S_{\text{MO}}(\%) \quad [4]$$

where $A_{z,t}$ is the signal intensity of by-product peaks, $A_{S,t}$ is the signal intensity of the internal standard, and N is the number of protons of the functional group ($N_{2.9} = 2$; $N_{3.1} = 2$). As in the case of oleic base species, the equation can be simplified because of the absence of by-products.

When results obtained by GC and ^{1}H NMR are compared for the epoxidation of methyl oleate and methyl linoleate, as shown in Table 1, similar values for conversion and selectivity are found, which proves the reliability of this method. The discrepancy between GC and NMR results for methyl linoleate (Table 1, entry 3) is due to the difference in sensitivity factors, which we were unable to determine. Because of the importance of reproducibility, five samples of epoxidized SUN and SAF were taken at the exact moment and analyzed by using ^{1}H NMR. In Table 2, for each group of samples the mean value is determined, as is the SD. Only small margins of error were found. No reproducibility tests were performed on GC.

ACKNOWLEDGMENT

Sponsorship of the research by N.V. Oleon (Belgium) is acknowledged.

TABLE 2
Reproducibility of Results[a] Using ^{1}H NMR

Substrate	Amount *m*CPBA (mmol)	t_{reaction} (min)	μ (S_{MO})	σ^2 (S_{MO})	μ (S_{DO})	σ^2 (S_{DO})	μ (X)	σ^2 (X)
SUN	3	8	94.14	0.21	5.86	0.21	54.50	0.25
SUN	3	12	94.62	0.22	5.35	0.17	51.30	0.26
SAF	6	10	63.20	0.16	36.80	0.16	57.75	0.33

[a]Five samples are taken at each time and analyzed by ^{1}H NMR. Conditions include 2 mmol substrate, 10 mL $CHCl_3$, *m*CPBA (70%), 293 K. μ, average; σ^2, SD; SUN, high-oleic sunflower oil; SAF, safflower oil; for other abbreviations see Table 1.

REFERENCES

1. AOCS, Iodine Value of Fats and Oils, Wijs Method, Revised 1992, *Official and Recommended Methods of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1993, Method Cd 1-25.
2. AOCS, Oxirane Oxygen, *Official and Recommended Methods of the American Oil Chemists' Society*, 3rd edn., American Oil Chemists' Society, Champaign, Method Cd 9-57, reapproved 1989.
3. Rüsch gen. Klass, M., and S. Warwel, Complete and Partial Epoxidation of Plant Oils by Lipase-Catalyzed Perhydrolysis, *Ind. Crops Prod. 9*:125–132 (1999).
4. Sonnet, P.E., and T.A. Foglia, Epoxidation of Natural Triglycerides with Ethylmethyldioxirane, *J. Am. Oil Chem. Soc. 73*: 461–464 (1996).
5. Gelbard, G., O. Brès, R.M. Vargas, F. Vielfaure, and U.F. Schuchardt, ^{1}H Nuclear Magnetic Resonance Determination of the Yield of the Transesterification of Rapeseed Oil with Methanol, *Ibid. 72*:1239–1241 (1995).
6. Mannina, L., A.P. Sobolev, and A. Segre, Olive Oil as Seen by NMR and Chemometrics, *Spectrosc. Eur. 15(3)*:6–14 (2003).
7. Chapman, D., and F.M. Goni, Nuclear Magnetic Resonance, in *The Lipid Handbook*, 2nd edn., edited by F.D. Gunstone, J.L. Harwood, and F.B. Padley, Chapmann & Hall, London, 1994, pp. 511–533.

[Received December 18, 2003; accepted August 6, 2004]