Soy Fatty Acid Oxidation with Sodium Hypochlorite Monitored by Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: The oxidation process of soy fatty acids by sodium hypochlorite with ruthenium trichloride catalyst was examined at different temperatures and active chlorine:fatty acid molar ratios. ^1H and ^{13}C distortionless enhancement by polarized transfer nuclear magnetic resonance (NMR) spectroscopy techniques were used to monitor oxidation of the double bonds in unsaturated lipids by measuring the peak integration ratio of double-bond peaks:methylene-methyl peaks. This NMR monitoring technique proved to be an excellent means to quantify double-bond reactions. Gas chromatography–mass spectrometry was used to identify mono- and diacid products, separated by hexane/methylene chloride extraction, as well as other oxidation products. While the presence of ruthenium catalyst increased the initial rate of oxidation, it also catalyzed the decomposition of hypochlorite, decreasing the available reactive chlorine, resulting in a delay in complete oxidation. A 9:1 molar ratio of active chlorine to fatty acids completely oxidized all double bonds of soy fatty acids. However, the yield of low-molecular-weight monoacid oxidation products was only 17%, indicating the probable formation of hydroxy fatty acids.
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KEY WORDS: Fatty acids, NMR spectroscopy, oxidation, ruthenium chloride, sodium hypochlorite, soybean oil.

Soybean fatty acids consist of more than 80% unsaturated long-chain fatty acids. Oxidative fissions provide a chemical way to cut these fatty acids into short-chain mono- and diacids. The diacids are important raw materials for synthetic polymers, such as polyesters and nylons, and the short-chain monoacids are useful materials in making cellulose ester plastics (1,2).

Ruthenium catalysts have previously been used for the oxidative cleavage of oleic acid with chlorine (3–5). Zaidman *et al.* (6) presented different oxidizing agents, including potassium permanganate, bichromate, chromic acid, hydrogen peroxide, ozone, and sodium hypochlorite, in the oxidative cleavage of oleic acid double bonds, analyzed their economic performance, and developed a manufacturing computer-simulation based on sodium hypochlorite-$RuCl_3$ oxidation technology. The economic parameters obtained showed that sodium hypochlorite is the most commercially efficient oxidant. For industrial oxidation processes, it is likely that a mixture of unsaturated fatty acids will be used, such as soybean oil. This paper presents results of a study on long-chain unsaturated fatty acid oxidation by sodium hypochlorite with ruthenium chloride catalyst. We also report the use of nuclear magnetic resonance (NMR) spectroscopy as a monitor to observe unsaturated fatty acid oxidation, and present results on the separation of mono- and diacids by solvent extraction.

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MATERIALS AND EXPERIMENTAL METHODS

Distilled soybean fatty acids were kindly provided by Karlshamns USA Inc. (Columbus, OH). Sodium hypochlorite (available chlorine content 4%), ruthenium trichloride, and boron trifluoride–methanol complex (~50 wt% BF_3) were purchased from Aldrich Chemical Company (Milwaukee, WI). All other materials were of reagent grade.

The NMR spectra were recorded with a Bruker ARX300 spectrometer (Burlington, Ontario, Canada). The oxidized fatty acid samples were prepared in $CDCl_3$ and referenced to tetramethylsilane (TMS) as an internal standard. 1D ^1H, 2D ^1H-^1H COSY (7) and ^{13}C DEPT (distortionless enhancement by polarized transfer) NMR (8) techniques were used to monitor the oxidation reaction (^{13}C NMR conditions: 0.49 s acquisition time, D_1 = 2.0 s, 200 scans; ^1H NMR conditions: F_2-acquisition parameters: pulse sequence correlation spectroscopy (COSY) 45, number of points = 1024, 16 scans, spectral width 1766 Hz; F_1-acquisition parameters: number of points = 128, number of increments = 64, spectral width 1766 Hz). The gas chromatography–mass spectrometry (GC–MS) results were obtained on a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA) and Finnigan 4000 mass spectrometer (San Jose, CA). GC analyses were run on a 0.25 mm i.d. × 30 m, 0.25 μm DB1 column (J&W Scientific Products, Folsom, CA) with a temperature program from 50 to 280°C at a rate of 15°/min. A standard titration procedure was used to measure available chlorine content (9). All measurements were performed in duplicate on each sample.

Oxidation reaction conditions. Soybean fatty acids (12.5 g, 45 mmole) were dissolved in 20 mL hexane and placed into a round-bottom flask (1000 mL capacity) that contained 10

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mL of 1 N sodium hydroxide solution. Appropriate amounts of sodium hypochlorite (90, 112.5, 135, 202.5 mmoles) were added with vigorous mechanical stirring. After 40 min, an equivalent amount of sodium hypochlorite solution was added to make total chlorine:fatty acid molar ratios of 4:1, 5:1, 6:1 and 9:1. In the catalyzed reactions, 3 mg ruthenium trichloride was also added. The reaction solution pH was maintained at 12.5 (as followed by pH indicator paper) throughout the entire reaction process by the addition of sodium hydroxide solution. The reaction was terminated by the addition of 1 N sodium thiosulfate solution to reduce any remaining active chlorine, followed by acidification to pH 2.5 with concentrated HCl. Three extractions, with 250 mL hexane each time, were used to isolate hexane-soluble oxidized fatty acid products. The aqueous phases were combined and further extracted with three extractions, with 250 mL methylene chloride, to obtain methylene chloride-soluble oxidized fatty acid products.

Samples were taken during the reaction for analysis of active chlorine, proton/carbon NMR, and GC–MS. NMR and GC–MS samples were prepared for analysis by first reducing residual active chlorine with sodium thiosulfate, acidifying to pH 2–3, and then extracting sequentially with hexane and methylene chloride. The hexane and methylene chloride phases were combined, and after removing solvents by evaporation, the oxidized fatty acids were analyzed.

Methylation of oxidized fatty acids for GC–MS analysis (10). About 1.2 g of oil samples was added in a round-bottom flask (50 mL capacity) with a magnetic stir bar and 12 mL methanol and 0.3 g potassium hydroxide. The mixture was boiled and stirred under refluxing conditions for 3 to 5 min to dissolve the potassium hydroxide. The mixture was cooled to 40°C, and 1.7 g of boron trifluoride–methanol complex was added. The mixture was heated again to the refluxing temperature and maintained for 6 min. The heating was stopped, and 10 mL isooctane and 24 mL saturated sodium chloride solution were added. The solutions were mixed in a separatory funnel by agitation for 15 s, and the organic phase was isolated, collected, and dried over magnesium sulfate. The isooctane solvent was removed by evaporation at reduced pressure in a flowing nitrogen stream, yielding 1.1 g of fatty acid methyl esters.

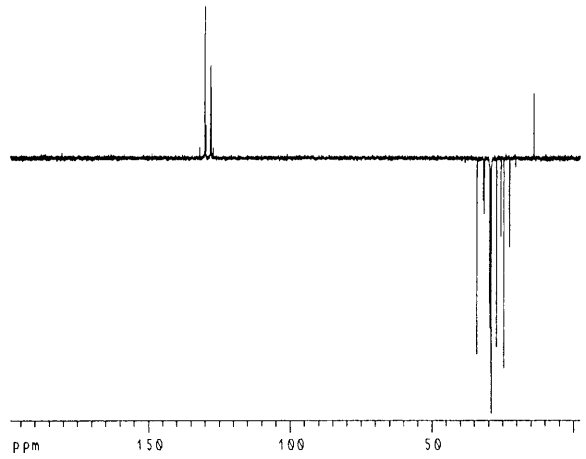

FIG. 2. The distortionless enhancement by polarized transfer (DEPT) ^{13}C NMR spectrum of soy fatty acids. Methyl and methine peaks point upward, methylene peaks point downward.

RESULTS AND DISCUSSION

The general fatty acid composition of soybean oil is palmitic acid 10%, stearic acid 4%, oleic acid 26%, linoleic acid 52%, and linolenic acid 8% (11). Based on this composition, the average molecular weight of the soy fatty acids is 278.58 Daltons with 1.53 moles of double bonds per mole of fatty acid, as calculated from the molar composition of the constituent fatty acids.

Proton, carbon (DEPT), and 2D ^{1}H-^{1}H COSY NMR spectra of unoxidized soy fatty acids are presented in Figures 1–3. In Figure 1, seven groups of peaks are present,

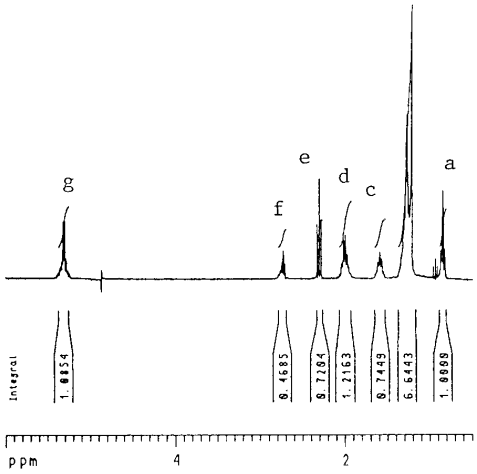

FIG. 1. The ^{1}H nuclear magnetic resonance (NMR) spectrum of soy fatty acids. Designations Ha-Hg refer to proton assignments (see Figs. 3 and 4, and text).

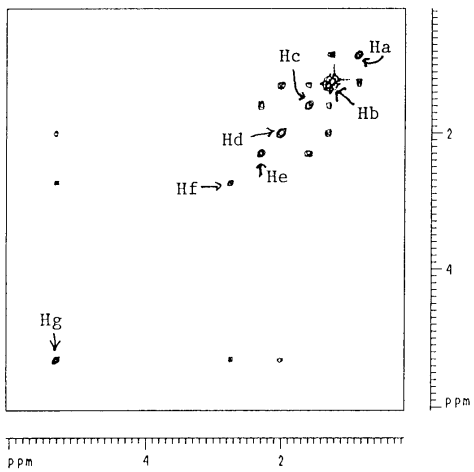

FIG. 3. The 2D ^{1}H-^{1}H correlation spectroscopy (COSY) NMR spectrum of soy fatty acids. Designations of Ha-Hg peaks refer to proton assignments (see Figs. 1 and 4, and text). See Figure 1 for abbreviation.

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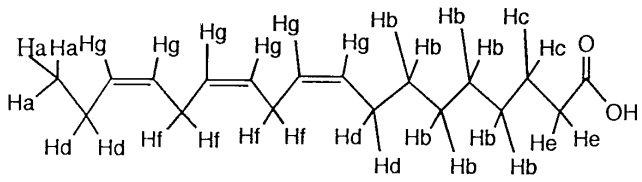

FIG. 4. The assignment of unsaturated fatty acid protons based on COSY NMR. See Figures 1 and 3 for abbreviations.

designated Ha-g. The first group of peaks (Ha), at 0.82–0.87 ppm, are from the terminal methyl protons of the fatty acids. The last group of peaks (Hg), at ~5.3 ppm, are from double-bond protons. The carbon (DEPT) spectrum in Figure 2 shows the methyl and methine carbon peaks, observed at 14.1 and 127–131 ppm, pointing upward, and methylene carbons, at 22–34 ppm, pointing downward. The ^{1}H-^{1}H COSY spectrum is shown in Figure 3. By tracing cross peaks, structural assignments can be made. The peaks (Hf) at ~2.7 ppm are resonance signals from the protons between double bonds from linoleic and linolenic acids, as shown in Figure 4. The evidence for this assignment is that from the cross peaks, in which Hf protons only correlate with double-bond protons, Hg. The peaks (He) at 2.2–2.3 ppm are from the protons at the alpha positions of the carboxylic carbon atoms, because these protons only correlate with methylene protons (Hc) once at the high frequency side. The peaks (Hd) at 1.9–2.0 ppm are from the protons at the alpha positions of double bonds, but not those between double bonds. This assignment is based on the fact that they correlated with both double-bond protons (Hg) and methylene (Hb) protons (Fig. 4). The Hc group of peaks are methylene protons adjacent to He protons, because they correlate with both He and Hb. Finally, the Hb group of peaks are methylene protons between Hd and Hc.

Monitoring oxidation by NMR. Because there are approximately 1.53 double bonds per mole of soybean fatty acids on average and 2 protons on each double bond, there are approximately 3 moles of double-bond protons per mole of soybean fatty acid. This is equivalent to the number of moles of terminal methyl protons per mole of soybean fatty acids. Comparing the peak integrations of the double-bond protons (peaks Hg at 5.3 ppm in Fig. 1) and methyl protons (peaks Ha at 0.82–0.87 ppm in Fig. 1), this is confirmed by nearly identical values of 1.08 and 1.0 (integration values given below each set of peaks). As oxidation occurs, the methyl protons (Ha) are not reactive, and therefore the Ha signal remains unchanged. However, the double-bond peak signal decreases as oxidation occurs, so the ratio of the integration peak values of the double-bond protons:methyl protons can be used to monitor the fatty acid oxidation process by ^{1}H NMR.

Effect of hexane solvent on fatty acid oxidation. Mass transfer is an important factor to affect reaction rate. Initially, a 4:1 molar ratio of active chlorine:fatty acids was reacted at 50°C for 24 h with ruthenium catalyst in the absence of hexane solvent. After 24 h, no active chlorine was found in the reaction mixture. ^{1}H NMR showed that about 31% of the double-bond protons were left (Fig. 5). We also observed that, during the reaction, solid particles of lipids suspended in the aqueous phase were formed. To avoid this problem, the fatty acids were dissolved in hexane prior to the addition of sodium hypochlorite solution. Under the same reaction conditions, only about 17% double-bond protons remained (Fig. 6). This improved reaction yield is proposed to be due to the improved mass transfer between the fatty acids dissolved in hexane and the hypochlorite in the aqueous phase.

Oxidation products. Molar ratios of active chlorine:fatty acid of 4:1, 5:1, and 6:1 were not adequate to completely oxidize the fatty acids, as evidenced by the presence of Hg protons in the product. However, at a 9:1 molar ratio, fatty acids, reacted for 3 d at 25°C with ruthenium catalyst, showed no double bonds by ^{1}H NMR analysis of the hexane-soluble oxidation products (Fig. 7). The spectrum became much simpler than that of the reactant fatty acids, with all peaks related to

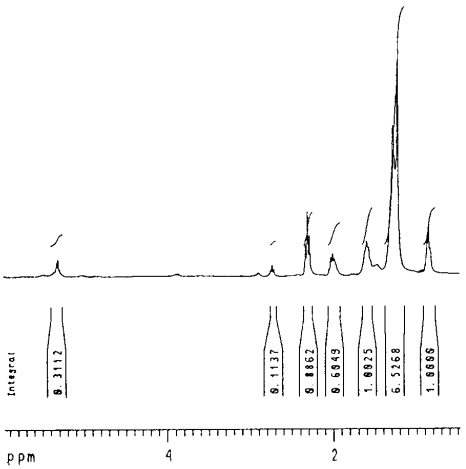

FIG. 5. The ^{1}H NMR spectrum of oxidized soy fatty acids without the use of hexane. The peak at 5.3 ppm correlates to the presence of double-bond protons. See Figure 1 for abbreviation.

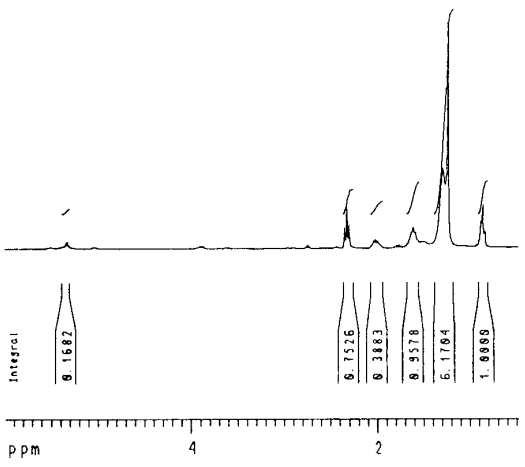

FIG. 6. The ^{1}H NMR spectrum of oxidized soy fatty acids with the use of hexane. The peak at 5.3 ppm correlates to the presence of double-bond protons. See Figure 1 for abbreviation.

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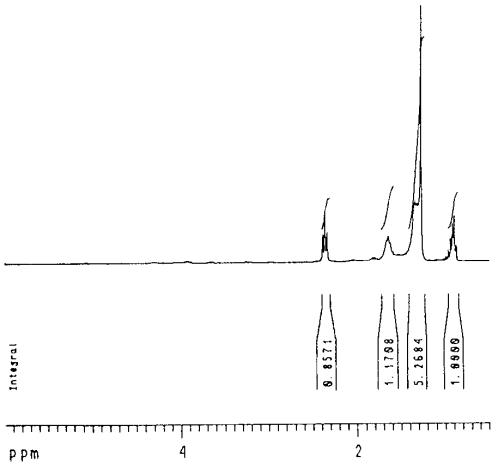


FIG. 7. The ^{1}H NMR spectrum of oxidized soy fatty acids with 9:1 molar ratio of active chlorine:fatty acids, 25°C, 3 d. The absence of a peak at 5.3 ppm indicates the absence of double-bond protons. See Figure 1 for abbreviation.

double bonds (peaks Hg, Hf, and Hd) having vanished. The hexane-soluble oxidized fatty acid products were isolated at reduced pressure (0.5 mm Hg, 40°C), to yield approximately 17% low-molecular-weight monoacids, much less than the theoretical yield. Based on the GC–MS analysis results, most of the products have higher molecular weights than palmitic and stearic acids (data not published). ^{13}C NMR analysis of these materials showed chemical shifts in the range of 52 to 76 ppm and were confirmed as methine peaks by DEPT NMR (Fig. 8).

While the structure of these products is not known, we believe that these materials are hydroxylated fatty acids. The ^{13}C NMR chemical shift values were calculated for theoretical hydroxylated fatty acids (12) and were compared to obtained spectra. The basic value of methylene carbon in fatty acids is about 22 ppm, and the ^{13}C shielding parameters ($\Delta\delta$) of the hydroxyl groups, connected directly and in the alpha position to the methine carbon, are 41 and 8 ppm, respectively. Therefore, the chemical shift of hydroxylated carbon of 1,2 diols is about 22 + 41 + 8 = 71 ppm, similar to the peaks observed by NMR analysis. Chemical shifts for 1,2,4,5-tetraols can be calculated in a similar fashion (12) and give values of 66 ppm or lower. While chemical shifts for hexaols are too complicated to calculate reliably, their chemical shifts should also lie in the 50 to 60 ppm range. Thus, the observed multiple peaks in the 52 to 76 ppm range are believed to be a complex mixture of 1,2 diols, 1,2,4,5-tetraols, and 1,2,4,5,7,8-hexaols of hydroxylated fatty acids.

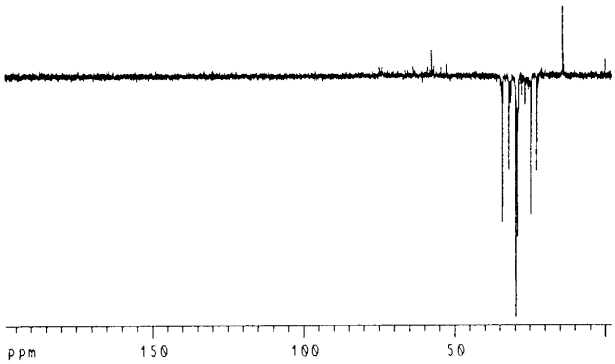
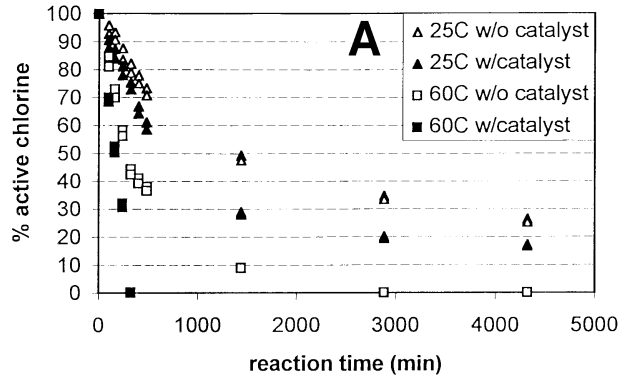


FIG. 8. The ^{13}C DEPT NMR spectrum of oxidized, fractionated soy fatty acids with 9:1 molar ratio of active chlorine:fatty acids, 25°C, 3 d. See Figures 1 and 2 for abbreviations.



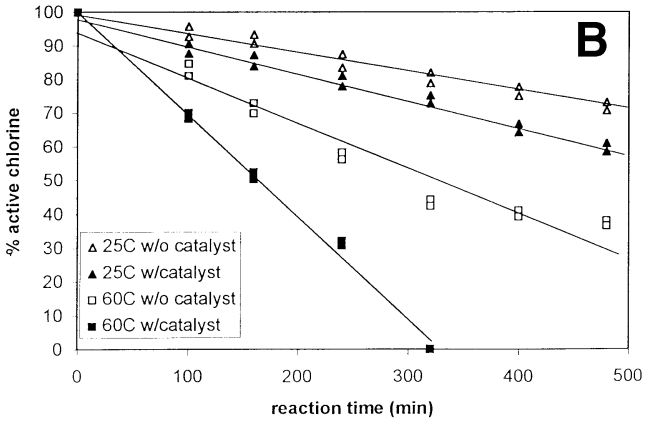


FIG. 9. Chlorine consumption vs. reaction time. (A) Entire data range shown; (B) data at $t < 8$ h; lines are unweighted linear regression fits to data.

GC–MS analysis of the methylated hexane- and methylene-chloride extracted samples showed effective separation of mono- and diacids. In the hexane extraction, only C_6–C_9 monoacids were detected, along with higher-molecular-weight products (palmitic, stearic, and probably hydroxy stearic acids). In the methylene chloride soluble products, only C_8 and C_9 diacids were found. Therefore, the hexane/methylene chloride extraction process used here is an effective means to separate mono- and diacids from fatty acid oxidations.

TABLE 1
Initial Reaction Rate for Active Chlorine (%) vs. Time for 0–8 h

	Slope	r^2 (correlation coefficient)	Standard error
25°C w/o catalyst	–0.0597	0.987	1.651
25°C w/catalyst	–0.0817	0.992	1.717
60°C w/o catalyst	–0.137	0.972	5.608
60°C w/catalyst	–0.3050	0.998	2.627

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Active chlorine consumption vs. reaction time. The available active chlorine in the reaction mixtures was measured vs. reaction time at 25 and 60°C, with and without ruthenium trichloride. The results are presented in Figure 9. The initial reaction rate data (0–480 min) were modeled by unweighted linear regression (Table 1), indicating that the presence of ruthenium catalyst increases the initial reaction rate by approx. 37% at 25°C and by 120% at 60°C. Between 25 and 60°C, the initial reaction rate was roughly doubled in the absence of catalyst and was tripled with catalyst. Attempts to fit first- and second-order reaction models in chlorine concentration to the data over the entire reaction time range resulted in poor model fits (results not shown).

Double-bond conversion vs. reaction time. As oxidation occurs, the number of double-bond protons in soy fatty acids decreases, as measured by ^{1}H NMR (Fig. 10). The initial rate of reaction was modeled at 25°C and at 60°C in the absence of catalyst by unweighted linear regression over the time range of 0 to 9 h (Table 2). The uncatalyzed initial reaction rate roughly doubled with increased temperature. In the presence of catalyst, the initial rate of reaction was significantly faster, and the reaction was basically completed when the first samples were taken. Based on the initial data points to estimate the initial reaction rate, the catalyzed reaction rate must be at least 3–5 times greater than the uncatalyzed rate. Attempts to model these reactions by first- and second-order models produced poor fits to the data values (not shown).

The data show that the ruthenium catalyst accelerates the initial double-bond oxidation rate but consumes more active chlorine and retards the completion of oxidation. Comparing Figures 9 and 10, at 25°C with catalyst, 48 h are required to react all double bonds, consuming 80% of the active chlorine. However, at 25°C without catalyst, only 24 h are required to react all double bonds, consuming only 51.4% of the active chlorine (close to the stoichiometric amount required). Thus, the absence of catalyst decreases the consumption of active chlorine by 31.4% and completes the oxidation faster. Similar results were obtained for the reaction at 60°C. Interestingly,

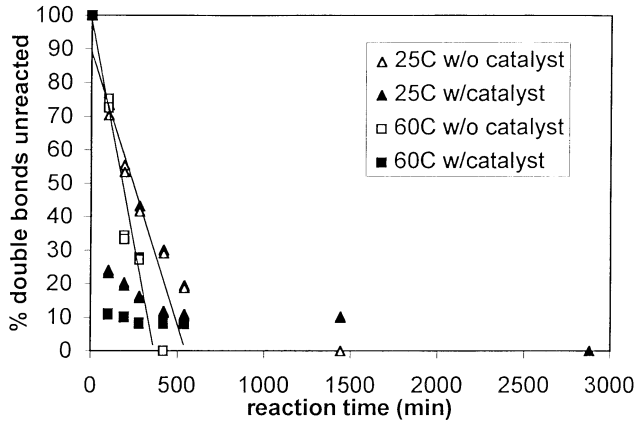

FIG. 10. Double-bond oxidation vs. reaction time; lines are unweighted linear regression fits to data at $t < 9$ h for uncatalyzed reactions.

TABLE 2
Initial Reaction Rate Data for Double Bonds vs. Time for 0–9 h, Without Catalyst

	Slope	r^2 (correlation coefficient)	Standard error
25°C	–0.1422	0.967	7.585
60°C	–0.2773	0.971	8.175

the complete oxidation of double bonds was delayed by the use of catalyst. One explanation for this is that, although the catalyst helps oxidation of double bonds, it also facilitates the decomposition of hypochlorite into hydrogen peroxide and sodium chloride. If the decomposition reaction competes with the double-bond oxidation reaction, the active chlorine concentration may be sharply reduced during the latter stages of the oxidation reaction, resulting in the delayed completion of oxidation.

During the reaction, several unexpected fatty acids were detected. While oxidative cleavage would only result in C_9 acids, C_8 and C_7 monoacids and C_8 diacid oxidation products were detected by GC–MS analysis (data not shown). The reactions leading to the formation of these acids are unknown. One possible explanation could be decarboxylation; however, no quantitative data are available to provide definitive proof that the use of ruthenium catalyst may also possibly lead to decarboxylation of unsaturated fatty acids during the oxidation process.

In conclusion, the use of ^{1}H NMR has proven to be useful for monitoring fatty acid double-bond cleavage. While it does not differentiate between products formed, it can be used to measure the molar amounts of double bonds present relative to methyl groups. While the use of ruthenium catalyst dramatically accelerates the initial oxidation of fatty acid double bonds, it also rapidly decomposes sodium hypochlorite. This results in higher chlorine consumption and longer reaction times for complete oxidation. While complete oxidation of the double bonds is achievable, the resulting products are a mixture of shorter-chain mono- and diacids as well as hydroxylated long-chain fatty acids. Some decarboxylation of the fatty acids also occurs. The use of hexane and methylene chloride extractions is a good analytical technique for separating mono- and diacid oxidization products.

ACKNOWLEDGMENTS

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