

Mechanism of Volatile Compound Production during Storage of Sunflower Oil

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Volatile compounds formed in the course of the thermal decomposition of hydroperoxides during storage of sunflower oil were analyzed by headspace solid-phase microextraction sampling followed by gas chromatographic separation and mass spectral detection. The determining role of alkoxy radicals in the process has been proven by electron spin resonance spectroscopic measurements. On the basis of analytical results, the reaction networks and mechanisms were constructed by computer modeling to describe the formation of volatile products of radical decomposition of hydroperoxides. We established that off-flavor aliphatic aldehydes are originated from only the alkoxy radicals derived from triglyceride of linoleic acid. To find a specific additive, which redirects the formation of these radicals toward production of more stable species, is suggested.

Keywords: Sunflower oil; volatile compounds; gas chromatography–mass spectrometry; electron spin resonance spectroscopy; reaction mechanism

INTRODUCTION

One of the main objectives of food chemistry is to avoid the deterioration of shelf-stored nutrition products. Many types of foodstuff, for example, edible oil-based spreads, must conserve their taste and flavor for several months. During storage, hydroperoxides are formed in vegetable oils in the presence of even a trace of oxygen. The off-flavor of oil is caused by the formation of volatile degradation products of thermolabile hydroperoxides (Schiberle et al., 1979; Schiberle and Grosch, 1981; Frankel et al., 1987) formed during the long-time storage of oils. The initial step of decomposition of fatty acid hydroperoxides is their homolytic breakdown into alkoxy radicals (Hopin et al., 1996; Davies and Slater, 1986; Emanuel and Gál, 1986).

In our earlier works (Keszler et al., 1998, 1999), we thoroughly investigated the possibilities of scavenging the alkoxy radicals using different additives in sunflower oil. Neither the usual phenol-type antioxidants (Emanuel and Gál, 1986), nor foodstuff additives (e.g., α -tocopherol and β -carotene) affected the accumulation of hydroperoxide degradation products.

Under acidic conditions ion-catalyzed degradation also occurs, yielding the same products, only in different amounts compared with the radical mechanism (Hopin et al., 1996).

We oxidized the sunflower oil and decomposed the formed hydroperoxides. The degradation products were identified by gas chromatography (GC)–mass spectrometry (MS) and electron spin resonance (ESR) spectroscopy.

The present investigations aimed to treat experimental data obtained by analysis using a computer program. This procedure consists of automatic generation of all stoichiometrically possible degradation reactions (possible mechanism), and their reduction based on chemical

and thermochemical criteria to clarify which way the experimentally found volatile components are formed. Knowledge of the reaction mechanism makes it possible to find the proper additives to keep the taste and flavor of sunflower oil.

Sunflower oil, a mixture of triglycerides, was considered too complex (numerous reactions) to be handled by the computer program. Therefore, we selected the hydroperoxide of linoleic acid (the main constituent of esters of sunflower oil) as a model to reveal reaction channels for the formation of volatiles.

EXPERIMENTAL PROCEDURES

Materials. Sunflower oil was refined in the same way as for commercial use by Unilever Research Laboratory, Vlaardingen (URL). 2-Heptenal, 2-octenal, 2-nonenal, 2,4-nonadienal, and 2,4-decadienal were synthesized also by URL; and hexanal, heptanal, and nonanal were obtained from Aldrich; the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) was from Sigma, and the chlorobenzene used as a solvent was from Fluka.

Oil Sample Preparation. Oil (100 mL) was oxidized by bubbling it with oxygen (1 bubble/s) at room temperature for 3 days, until the peroxide value (POV) determined by iodometric titration (Hendricks et al., 1979) had achieved a value of 110 mequiv/kg. POV is a measure of the active oxygen content, that is, the amount of hydroperoxides, peracids. For the GC–MS measurements 1 mL of oil samples were placed in glass tubes [5 mm inside diameter (i.d.) \times 250 mm] and deoxygenated in six repeated “freeze–vacuum–thaw” cycles. Before the thawing steps the tubes were filled with pure oxygen-free nitrogen. Finally, the tubes were sealed and conditioned at 120 °C for 120 min. The volatile compounds produced were collected using a solid-phase microextraction (SPME) sampler (Zhang and Pawliszyn, 1993) equipped with 100 μ m poly(dimethyl-siloxane) fiber coating. The optimal conditions (Keszler and Héberger, 1999) were: oil/headspace ratio, 1:1; 30-min equilibrium time at 40 °C; 1 min desorption at 220 °C. The use of enough narrow (0.75 mm) inlet liner enabled us to obtain fairly sharp peaks even in the early part of the chromatogram.

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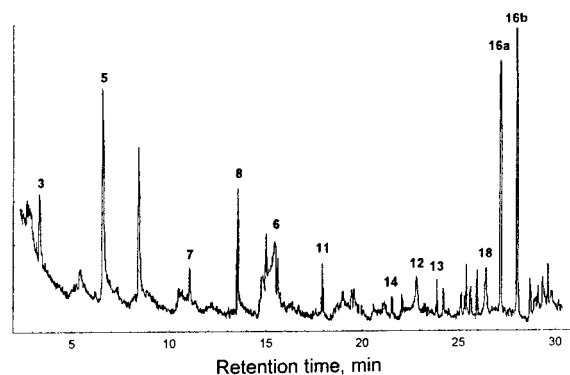


Figure 1. Total ion chromatogram of sunflower oil of POV = 110 after 120 min storage at 120 °C taken by headspace SPME sampling. 3, pentanal; 5, hexanal*; 7, heptanal*; 8, 2-heptenal*; 6, hexanoic acid; 11, 2-octenal*; 14, 2-nonenal*; 12, octanoic acid; 13, 2,4-nonadienal*; 18, decanoic acid; 16a, *trans,cis*-2,4-decadienal; 16b, *trans,trans*-2,4-decadienal*.

The ESR samples, which contained 10 mM DMPO dissolved in oil-chlorobenzene (20:80, v/v), were deoxygenated as described above. Spectra were taken immediately after heating, without opening the tubes.

Both ESR and GC-MS analyses were performed using 5–5 parallel samples.

Capillary GC-MS. The analysis was performed using a Finnigan MAT GCQ GC-MS apparatus. The separation was performed on a DB 5 MS (J&W Sci. Inc., Folsom, CA) column, 30 m × 0.25 mm i.d., and 0.25- μ m film thickness. The column temperature was programmed from 40 °C with a 4 °C/min increase rate up to 160 °C (hold for 5 min), followed by a 20 °C/min increase rate up to 210 °C (hold for 10 min). The carrier gas was helium with 35 cm/s constant linear velocity. Electron impact (EI⁺) mass spectra were taken at 70 eV; the temperatures of the ion source and of the transfer line were 160 °C and 220 °C, respectively.

ESR Spectroscopy. ESR measurements were made with a Magnetech model CU4 modular computer-controlled spectrometer. The spectra of spin adducts were recorded at the following conditions: microwave power, 18 mW; modulation frequency, 100 kHz; modulation amplitude, 1.76 G; scan time, 120 s. The hyperfine coupling constants of DMPO spin adducts were determined from the simulated model of measured spectra using the computing "Program EPR" (Rockenbauer and Korecz, 1996) developed at the Technical University of Budapest, Hungary.

Computer Modeling Program. The computer modeling program used in the implementation of the theoretical considerations is detailed in the literature (Nemes et al., 1977a–c). The input is the chemical composition of reactants and products and their basic thermochemical data. The latter were estimated with the help of the THERM (Ritter and Bozelli, 1991) and THERGAS (Muller et al., 1995) programs. All stoichiometrically possible elementary reactions leading to the formation of degradation products are generated (possible mechanism). The great number of reactions forming the possible mechanism has to be reduced. The reduction is based on the number of chemical bonds formed and on the number of changes in bond order between the various pairs of atoms in an elementary reaction, as well as on thermochemical considerations involving chosen limits in reaction enthalpy and free energy. The resulting reduced possible mechanism already treatable and can be summarized graphically in sequence networks, which show the possible transport of a chosen atom from species to species, resembling the usual reaction schemes.

RESULTS AND DISCUSSION

According to the analysis made at URL, the sunflower oil used contained 95% of triglycerides. Of them 66.3% were linoleic acid esters. Only the 0.7% of

Table 1. Volatile Compounds Analyzed in Sunflower Oil^a and in Its Model Compound^b

No	Species	Oil	Model	Structure
1	pentane		+	
2	pentanol		+	
3	pentanal	+	+	
4	pentanoic acid		+	
5	hexanal	+	+	
6	hexanoic acid	+	+	
7	heptanal	+	+	
8	2-heptenal	+	+	
9	2-heptanon		+	
10	heptanoic acid		+	
11	2-octenal	+	+	
12	octanoic acid	+	+	
13	2,4-nonadienal	+		
14	2-nonenal	+	+	
15	2-decenal	+	+	
16	2,4-decadienal	+	+	
17	2-undecenal	+		
18	decanoic acid	+		

^a Present work. ^b Analyzed by de Groot (1997).

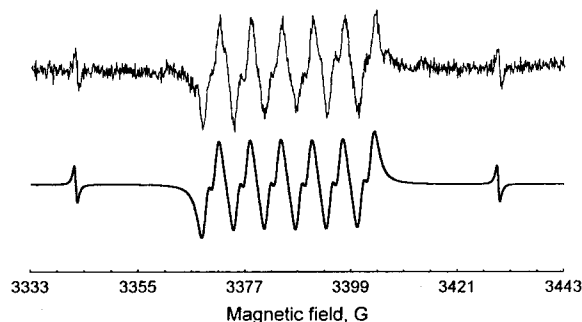


Figure 2. Measured and simulated ESR spectra of DMPO adducts of alkoxy radical taken during the decomposition of hydroperoxides present in sunflower oil of POV = 110, after 1 min storage at 120 °C.

constituents were polyunsaturated triglycerides. The remaining 32.0% consisted of stearic and oleic acids, the carbon chain of which is more resistant against oxidation than conjugated dienic chain (Kerr and Trotman-Dickenson, 1978). The latter explanation is supported by the findings of Sanchez-Muniz et al. (1994) that oleic acid concentration in fried sunflower oil remained unaltered, whereas the amount of linoleic acid decreased significantly.

In analyzing the untreated oil, no volatile production was observed. Therefore we oxidized the sunflower oil, then decomposed the formed hydroperoxides to imitate the aging. Then, we identified the degradation products and constructed the input matrix for computer modeling.

The input matrix was compiled from the following sources.

1. Volatile products, shown in Figure 1, were found in sunflower oil after oxidation followed by 120 min

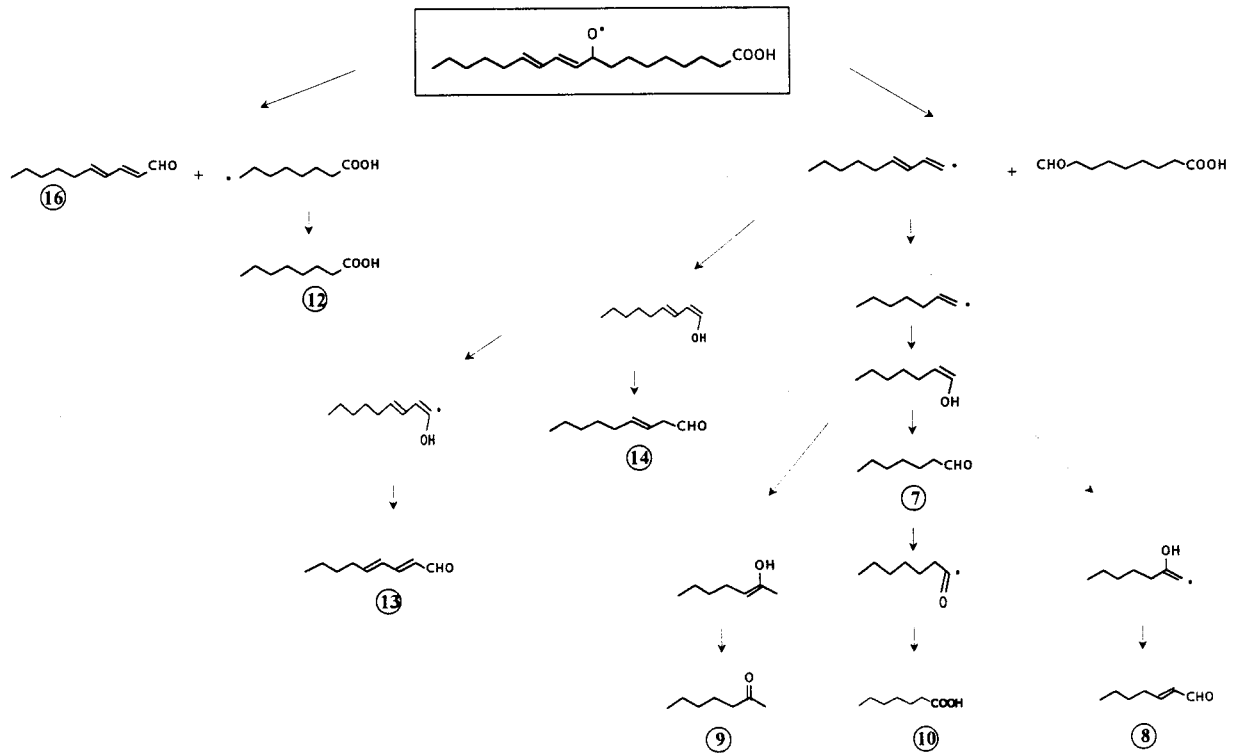


Figure 3. Carbon-sequence network of products derived from alkoxy radical of 9-linoleic acid. (Number identifies species as in Table 1.)

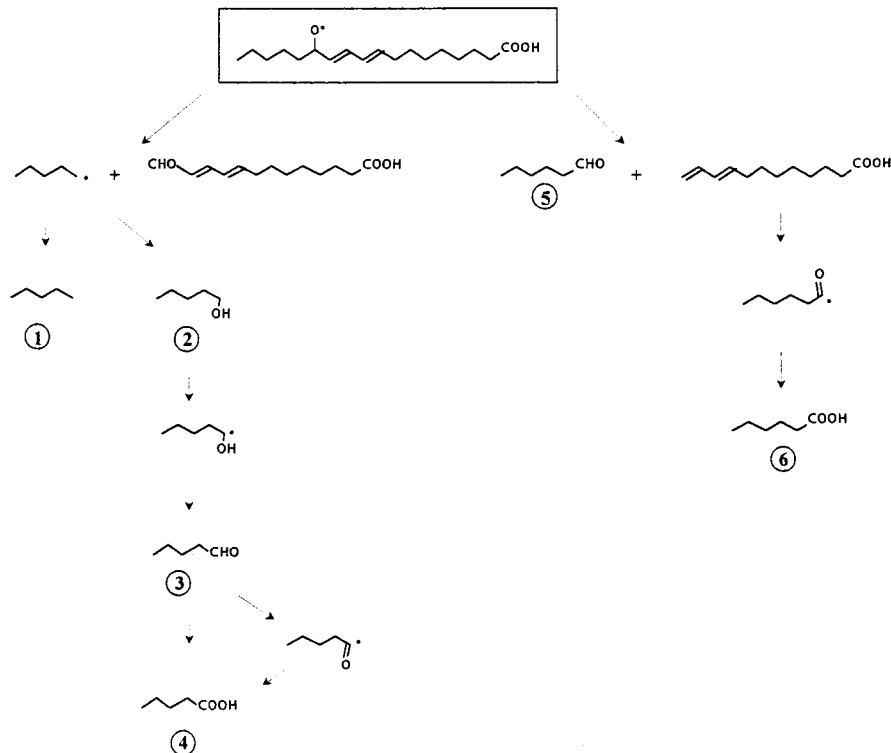


Figure 4. Carbon-sequence network of products derived from alkoxy radical of 13-linoleic acid. (Number identifies species as in Table 1.)

storage at 120 °C in sealed ampullae. All components were identified by matching their mass spectra with National Institute of Standards and Technology (NIST) spectral library (Finnigan Corp., San Jose, CA). Asterisks on the chromatogram indicate additional identifications using pure standard compounds. Some compounds were found during the decomposition of 9- and 13-linoleic acid hydroperoxide (prepared by enzymatic

oxidation of linoleic acid) with the purge-and-trap technique by de Groot (1997). The free radical oxidation of linoleic acid leads to formation of 9- and 13-linoleic acid hydroperoxide as well (Yamamoto et al., 1982). Volatile products obtained by both methods are collected in Table 1. The most aldehydes found are indicators of rancidity and give the oil a bad flavor (Wyatt, 1987; Marsili, 1984; Bravo et al., 1992).

2. Stable nonvolatile species were compiled based on Gardner's review (1987).

3. Radicals possibly participating in the decomposition of hydroperoxides without chain rupture were included according to the general scheme of Emanuel and Gál (1986).

4. Further radicals were compiled based on consideration of the following reaction types: radical displacement, combination, addition, elimination, disproportionation and intermolecular rearrangement. Formations of dimers and cyclic molecules were not taken into account.

Water, ethyn, pentanoic, hexanoic, heptanoic, octanoic, and nonanoic acids were considered only as products, whereas linoleic acid was considered as a reactant. To model oxygen-free atmosphere in the experiments, oxygen was considered only as a product.

The ESR spectra taken during the initial part of thermal degradation of hydroperoxides accumulated in sunflower oil at 120 °C showed the presence of the alkoxy radicals exclusively. Figure 2 displays the measured and simulated spectra of the alkoxy radical. Signals of manganese were used as internal standards for the determination of g -factor and can be seen on both sides of the measured signal. The g -factor of alkoxy radical was 2.008. The hyperfine coupling constants obtained from the simulation were as follows: $a^N = 12.85$, $a_\beta^H = 6.47$, and $a_\gamma^H = 1.88$. These constants are in good agreement with the data of Davies and Slater (1986), who investigated the decomposition of oleic, linoleic, linolenic, and arachidonic hydroperoxides.

The computer modeling results are summarized in the carbon skeleton sequence network of products (Figures 3 and 4). Because both 9- and 13-linoleic acid hydroperoxides are formed during oxidation of linoleic acid, different sequence networks have been computed for each isomer. Alkoxy radicals, the primary intermediates during decomposition, are the initial points of the graphs. The neighboring C–C bonds of alkoxy radicals readily undergo rupture. Taking into consideration the left and right positions of these β -scissions, two routes of product formation can be distinguished in both graphs. Figure 3 shows the fate of 9-linoleic acid alkoxy radical. The endproducts of the left direction are 2,4-decadienal and octanoic acid, whereas in the other direction routes of formation for heptene, 2-heptenal, heptanone, heptanal, heptanoic acid, 2-nonenal, and 2,4-nonadienal are shown. In 13-linoleic acid, on one side, alkoxy radical pentane, pentanol, pentanal, and pentanoic acid, and on the other side, hexanal and hexanoic acid are the volatile components of the network, as shown in Figure 4.

The overwhelming majority of stable components identified in the headspace of degraded sunflower oil are the same as in the model compound (Table 1). This confirms that the obtained network is suitable for describing the formation of volatiles in sunflower oil. 2,4-Nonadienal, 2-undecenal, and decanoic acid have been identified in sunflower oil, but not during the decomposition of linoleic acid hydroperoxides. 2,4-Nonadienal is present in the compiled networks, suggesting the possibility of its formation from linoleic acid as well. The two latter ones presumably may derive from minor constituents of sunflower oil such as oleic acid ester.

In conclusion, we established that aliphatic aldehyde products responsible for the off-flavor of the sunflower

oil originated from one type of intermediate, the alkoxy radical derived from linoleic acid hydroperoxides. The "obvious" solution of using phenolic type antioxidants or lipid-soluble vitamins (A, E) in processes mediated by free radicals has not affected favorably the product pattern of this reaction (Héberger et al., 1999). Based on the reaction routes presented, we suggest looking for a specific additive, which redirects the formation of alkoxy radicals toward the production of more stable species.

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