

Changes of Lemon Flavor Components in an Aqueous Solution during UV Irradiation

Yuko Iwanami, Hideki Tateba,* Nobuko Kodama, and Katsumi Kishino

Ogawa and Company, Ltd., 6-32-9 Akabanenishi, Kita-ku, Tokyo 115, Japan

A lemon flavor composed of lemon oil, water (pH 6 phosphate buffer), and ethanol, and the lemon-flavored drink were irradiated with UV light. Citral (**1** and **2**), which is one of the most important components expressing the typical lemon-like odor, significantly decreased with *Z*–*E* isomerization and there appeared 2-(3-methyl-2-cyclopenten-1-yl)-2-methylpropionaldehyde (**6**), *trans*-1,3,3-trimethylbicyclo[3.1.0]hexane-1-carboxaldehyde (**7**), *cis*-1,3,3-trimethylbicyclo[3.1.0]hexane-1-carboxaldehyde (**8**), (1,2,2-trimethyl-3-cyclopenten-1-yl)acetaldehyde (**9**), α -campholenealdehyde (**10**), photocitral A (**3**), epiphotocitral B (**5**), and photocitral B (**5**). New compounds of aldehyde **9**, **6** and **10**, were newly identified as photoreaction products of citral. Limonene, terpinolene, and nonanal decreased, while *p*-cymene increased after UV irradiation. Other components, such as sesquiterpene hydrocarbons, citronellal, linalool, and terpineols, were only slightly changed. These results suggested that citral is a more UV-unstable component in lemon flavor and the photolysis of citral could affect other components in lemon flavor during UV irradiation.

Keywords: *Changes of lemon flavor components; photoreaction of citral*

INTRODUCTION

The lemon flavor composed of lemon oil, water, and ethanol is one of the most useful flavoring materials for various foods, such as drinks, candies, and cakes. However, it is well-known that the flavor often deteriorates with age because of various factors. Among those many factors, there are considered to be two major factors, heat and light. The effects of heat on the deterioration of lemon flavor have been reported in many papers. Terpene hydrocarbons are mainly changed to terpene alcohols by acid-catalyzed hydroxylation (Clark and Chamblee, 1992), and citral having typical lemon-like odor degrades due to cyclization and oxidation reactions (Kimura et al., 1983). However, few reports about the effect of light on lemon flavor, even on other citrus flavors, were found except reports on photo-oxidation (Schieberle and Grosch, 1989; Ziegler et al., 1991). As various drinks packaged in clear bottles containing less oxygen were generally manufactured and commercialized, it is very important to understand the effect light, especially UV-light, has on lemon flavor.

The experimental condition was carefully chosen to clearly understand the UV effects on flavor deterioration. Lemon oil is mainly composed of terpene hydrocarbons, such as pinenes, limonene, and terpinenes, and oxygen-containing compounds, such as nonanal, citronellal, linalool, and citral (**1** and **2**), many of which are main contributors to the odor of lemon flavor as recognized by aroma extract dilution analysis (AEDA) (Schieberle et al., 1988). The odorous change of the lemon flavor can be evaluated by quantitative analyses of these aroma components. Because most of these components in lemon flavor are easily degraded by heat in the presence of acid, the heat effects should be severely controlled.

The aim of this study is to clarify how the lemon flavor deteriorates in a drink system by UV irradiation under strictly controlled conditions.

EXPERIMENTAL PROCEDURES

Materials. Cold-pressed lemon oil (*Citrus limon*) from California (FCC grade) was used. The distilled lemon oil was prepared by steam distillation of the same lemon oil for the comparison test as mentioned later. Citral (Quest, The Netherlands) composed of geranial (**1**) and neral (**2**) (2:1) was purified up to 99% (GC) by column chromatography. α -Campholenealdehyde (**10**) was obtained from Hoechst AG. Geranial diethyl acetal (**11**) and neral diethyl acetal (**12**) were prepared according to the usual method (Liu et al., 1984). 2-(3-Methyl-2-cyclopenten-1-yl)-2-methylpropionaldehyde (**6**) was prepared from 1*R*(-)-fenchone obtained from Aldrich Chemical Co. in three steps according to the procedures described previously (Erman et al., 1986; Varech and Jacques, 1969; Delepine, 1924). The total yield of **6** was 4%; MS data are shown in Table 2; IR (gas phase) cm^{-1} 3050, 2968, 2803, 2693, 1735, 1464, 1381; NMR δ_{H} (CDCl_3) 1.00 (6H, s), 1.59 (1H, dddd, $J = 5.9, 8.0, 8.0, 13.7$ Hz), 1.73 (3H, br s), 1.97 (1H, dddd, $J = 6.7, 6.7, 9.3, 13.7$ Hz), 2.20 (2H, m), 2.89 (1H, m), 5.22 (1H, br s), 9.50 (1H, s).

UV Irradiation of Lemon Flavor and Its Drink. After a lemon oil (0.1 g) was mixed in a 100 mL of 0.05 M phosphate buffer (pH 6)/ethanol (35/65), by stirring vigorously, the upper oil layer was removed. The residual solution, containing 90% of essential oil, was purged with nitrogen gas, followed by sealing the solution into 10 separate 10 mL clear glass bottles. Half of these bottles were irradiated with UV light (≤ 400 nm; Toshiba FL20BLB \times 2 + FL20SE \times 2) from a 50 cm height for 4 days at 30 °C, while the other five bottles were wrapped with aluminum film to cut the light and allowed to stand at 30 °C for the same number of days. The unreacted citral content was determined by HPLC. The lemon flavor components were recovered from each solution by Porapak Q column chromatography (Shimoda et al., 1987). The recoveries of citral were 68–80%. Each component was identified by comparing the Kovats index and mass spectrum with those of the relevant standard sample. The amount of each component was calculated by computing the gas chromatography (GC) area against an internal standard (2-octanol, 50 μg). The typical components were injected under the same analytical conditions. Their response factors to the flame ionization detector (FID) were calculated as shown in Table 1. Quantitative data of other components were estimated by using the same response factors in Table 1. The lemon-flavored drink was provided as follows: After a lemon oil (4.2 g) was mixed in 100 mL of water/ethanol (35:65), by shaking vigorously for 15 min, it was stored

* Author to whom correspondence should be addressed.

Table 1. Response Factors of Main Aroma Components (FID)

component	response factor ^a	component	response factor ^a
limonene	0.8	neral	1.0
nonanal	1.1	α -terpineol	0.9
citronellal	1.4	geranial	1.0
linalool	0.9	geranyl acetate	1.5
β -caryophyllene	0.8	geraniol	1.4

^a GC area of internal standard (2-octanol)/GC area of each component.

Table 2. MS Spectral Data of Photoreaction Products (3–10)

compd no.	RI ^a	MS spectral data
3	1515	152 (2), 137 (10), 123 (100), 109 (20), 95 (40), 81 (70), 79 (20), 67 (36), 55 (22), 41 (38), 39 (30)
4	1492	152 (2), 137 (4), 123 (3), 109 (35), 95 (20), 82 (36), 81 (87), 70 (100), 69 (35), 67 (42), 55 (16), 41 (33), 39 (19)
5	1434	152 (5), 137 (75), 123 (85), 109 (70), 95 (45), 84 (40), 83 (65), 81 (100), 79 (40), 69 (80), 67 (40), 55 (50), 41 (70), 39 (41)
6	1442	152 (2), 137 (4), 123 (1), 109 (2), 91 (5), 81 (100), 79 (20), 65 (2), 53 (7), 41 (7), 39 (5)
7	1393	152 (7), 137 (15), 124 (10), 123 (100), 109 (10), 95 (20), 82 (60), 81 (90), 79 (25), 67 (30), 55 (15), 41 (24)
8	1402	152 (3), 137 (10), 124 (13), 123 (100), 109 (12), 94 (20), 82 (12), 81 (75), 79 (20), 67 (25), 55 (10), 41 (16)
9	1472	137 (2), 119 (4), 109 (35), 108 (100), 95 (30), 93 (57), 91 (17), 81 (22), 67 (30), 55 (10), 41 (15), 39 (13)
10	1497	152 (3), 137 (1), 119 (4), 109 (14), 108 (100), 95 (28), 93 (64), 91 (16), 81 (8), 67 (16), 55 (7), 41 (10), 39 (6)

^a RI, Kovat's retention index on DB-Wax column.

at -15°C for 30 min and centrifuged at 3000 rpm for 5 min. The upper oil layer was removed. The extract (0.4 g) from lemon oil was dissolved in water (400 mL). After filtration, the solution was packed in two separate 190 mL clear glass bottles, purged with nitrogen gas, and sealed with aluminum caps. The experiment of UV irradiation for the drink was carried out in the same condition as for the lemon flavor.

Photoreaction of Citral in Ethanol. Citral (1 g) was dissolved in 300 mL of ethanol, which was then irradiated with a 400 W high-pressure mercury lamp through a Pyrex tube under nitrogen. The reaction mixture was analyzed using GC and high-performance liquid chromatography (HPLC). Compounds 3–9 were isolated from a concentrate of the reaction mixture by column chromatography (*n*-hexane/ethyl acetate) and preparative GC.

GC. GC was carried out by using a Hewlett-Packard 5890 Series II gas chromatograph equipped with FID. Separation was achieved on a fused silica capillary column, 30 m \times 0.25 mm i.d., 0.25 μm film thickness (DB-Wax, J&W Scientific Inc.). The oven temperature was programmed from 80 to 200 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$. The temperatures of both injection and the detector were 250 $^{\circ}\text{C}$. The nitrogen gas flow rate was 50 mL/s, with an injection splitter having a split ratio of 200:1.

Preparative GC was carried out by using a Hewlett-Packard 5890A gas chromatograph equipped with a fused silica capillary column, 30 m \times 0.53 mm i.d., 1 μm film thickness (Supelcowax TM10, Supelco Inc.), and thermal conductive detector (TCD).

GC/Mass Spectrometry (GC/MS). Electron impact mass spectrometric data were collected by using a Hewlett-Packard 5971A mass spectrometer interfaced to a Hewlett-Packard 5890 Series II gas chromatograph. Separation was achieved on a fused silica capillary column, 60 m \times 0.25 mm i.d., 0.25 μm film thickness (Supelcowax TM10). The oven and injection temperatures were the same as those described for the GC

analysis. The helium gas flow rate was 1 mL/s, with an injection splitter having a split ratio of 50:1.

HPLC. HPLC analyses of the photodeterioration of lemon flavor and citral were performed by using a HP1090M instrument equipped with a photodiode array detector. The photolysis of citral was monitored by reversed-phase HPLC using a Capcell Pak C₁₈ SG120 (S-5 μm) column (Shiseido, 4.6 mm diameter \times 250 mm) with a constant elution gradient from 10% (v/v) acetonitrile in water to acetonitrile with a constant flow rate of 1 mL/min.

GC/Fourier Transform Infrared Spectroscopy (GC/FTIR). GC/FTIR data were collected on a Hewlett-Packard 5965B system interfaced to a Hewlett-Packard 5890 Series II gas chromatograph. Separation was achieved by using a fused silica capillary column, 60 m \times 0.25 mm i.d., 0.25 μm film thickness (DB-Wax, J&W). The oven and injection temperatures were the same as described for the GC analysis. The transfer line was held at 270 $^{\circ}\text{C}$. The helium gas flow rate was 1 mL/s, with an injection splitter having a split ratio of 50:1. Vapor-phase FTIR spectra were recorded from 750 to 4000 cm^{-1} with a resolution of 8 cm^{-1} .

Other Spectrometries. High-resolution MS was determined at 20 eV by using a Hitachi M80B spectrometer. NMR spectra were obtained in CDCl_3 with TMS as an internal standard on a Bruker AM400 instrument.

RESULTS AND DISCUSSION

UV Irradiation of Lemon Flavor and Its Drink.

Even though lemon flavors are often used in soft drinks together with lemon or other fruit juices that contain natural or added acids, the test of UV irradiation effect to lemon flavor was carried out in a higher pH test solution, pH 6 phosphate buffer/ethanol solution, to prevent the acid-catalyzed reactions. Actually, few acid-catalyzed reactions of lemon oil components were observed at pH 6 and room temperature.

The change of components in the lemon flavor was consistent with that in lemon-flavored drink. This change for lemon flavor corresponded with that for the lemon drink stored for 7 days at a distance of 10 cm from fluorescent light (12 000 lx). Figure 1a shows the results obtained by UV irradiation experiments of the lemon flavor. The component analyses were repeated five times for the lemon flavor. Quantitative data are expressed as mean values of the five results. The coefficient of variation (CV) was <23% for any data. Statistically significant data were calculated. Citral decreased rapidly with *Z*–*E* isomerization, and new peaks, such as I, II, and III, appeared. Monoterpene hydrocarbons, such as limonene and terpinolene, and nonanal decreased, while *p*-cymene increased. Other components, such as sesquiterpene hydrocarbons, citronellal, linalool, and terpineols, changed only slightly. The fresh, sweet, and typical lemon-like odor disappeared, while a dusty odor increased.

The original cold-pressed citrus oil has some of low-volatile or nonvolatile components, such as tocopherols (Ifuku and Maeda, 1978; Piironen et al., 1986), coumarins (Cieri, 1969) and other (Angelo et al., 1970). Those substances were confirmed to be extractable by water/ethanol solution from the cold-pressed oil. Therefore, those components were also considered to be the important factors for deterioration of lemon flavor. To confirm the effect of those components, the distilled lemon oil, which did not contain low-volatile or non-volatile components, was prepared from the original cold-pressed lemon oil by steam distillation, and its flavor was irradiated with UV light by using the same procedure as described previously. The terpene hydrocarbons and nonanal in the distilled oil solution tended

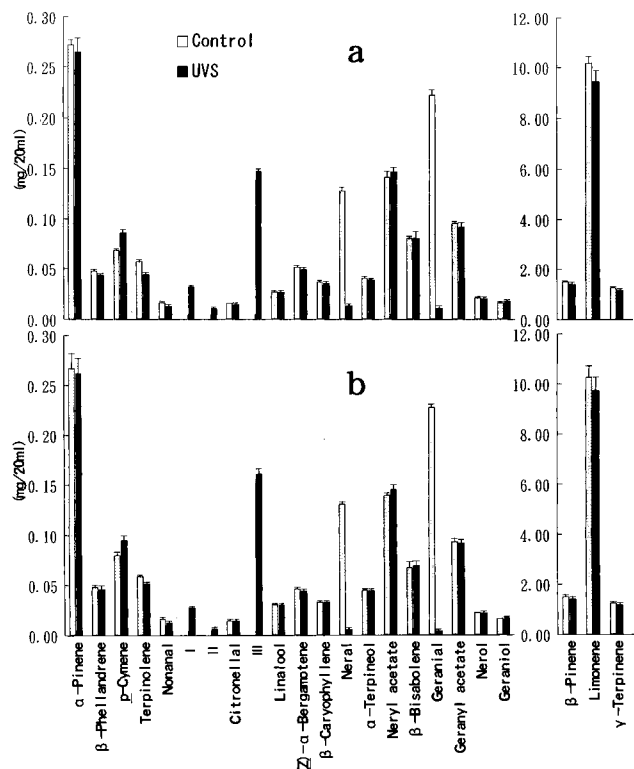


Figure 1. Changes of lemon flavor components: (a) lemon flavor made from original cold-pressed lemon oil; (b) lemon flavor made from distilled lemon oil. UVS, UV-irradiated flavor.

to similarly decrease as shown in Figure 1b. The differences in the amounts of the products were checked by using Mann–Whitney's U-test. A clear difference was observed in citral ($P < 0.05$). The subtle differences were indicated in octanal and terpinolene. Therefore, the low-volatile or nonvolatile components in lemon flavor have less effect on the volatile components except citral. Citral was recovered more from the reaction mixture of original cold-pressed lemon oil solution than from the reaction mixture of freshly distilled lemon oil solution. These results indicated that the volatile components except citral of original cold-pressed lemon oil solution should be only slightly affected with light due to the UV-sensitive components which were suggested to stabilize citral oppositely.

Photoreaction of Citral (1 and 2) in Ethanol.

Because citral, which is composed of 1 and 2 isomerizing rapidly, disappeared during UV irradiation on lemon flavor, we tried to investigate the photoreaction of citral. It was already reported that citral was converted to photocitral A (3), epiphotocitral A (4), and photocitral B (5) at room temperature (Cookson et al., 1963) and to the 1,2-migration products (7 and 8) of the formyl group at higher temperature ($\geq 80^\circ\text{C}$) (Wolff et al., 1980) in an aprotic solvent under nitrogen. The photoreaction of citral in a protic solvent except for 3–5 (Büchi and Wüest, 1965) has not been reported.

Citral in ethanol was irradiated by UV light under nitrogen, and the reaction products were analyzed. From the photolysis of citral, aldehydes of 7, 8, and α -campholenealdehyde (10) and also neral diethyl acetal (12) and geranial diethyl acetal (11) were produced in addition to the reported components of 3–5, as shown in Figure 2. 10–12 were identified by comparing the GC/MS data with those of standard samples. Photocitrals 3–5 and aldehydes 7 and 8 were identified by comparing the spectral data with those reported in the

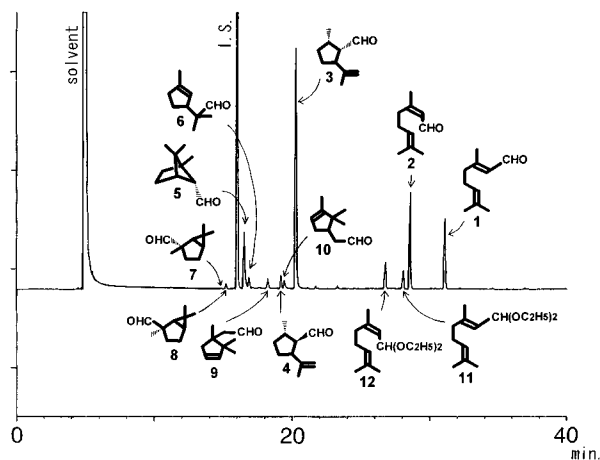


Figure 2. GC chromatogram of photoreaction products of citral in ethanol on DB-Wax.

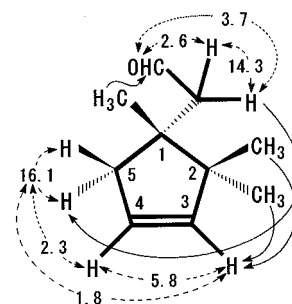


Figure 3. Structure of product 9. Lines show NOEs. Dotted lines show $J_{\text{H}}(\text{Hz})$ s.

literature (Kaiser and Lamparsky, 1976; Wolff et al., 1980). The structure of 6 was confirmed by comparing the spectral data of an authentic sample prepared by using the procedure mentioned under Experimental Procedures. In the photoreaction of citral, 6 was a new product, the formation of which requires the 1,3-migration of the formyl group. Small amounts of 7 and 8 were obtained at room temperature, and the yields increased at 80°C . Diethyl acetals (11 and 12) might be obtained from the hydrogen abstraction reaction of excited citral because 11 and 12 were not obtained in the dark under this condition. The structure of compound 9 was presumed on the basis of the various spectral data as shown in Figure 3. The high-resolution mass spectrum of 9 showed that the molecular formula of $\text{M}^+ - 15$ was $\text{C}_9\text{H}_{13}\text{O}$ (m/z calcd for $\text{C}_9\text{H}_{13}\text{O}$, 137.0966; found, 137.0944). GC/FTIR suggested the presence of a carbonyl group (1734 cm^{-1}) and methine (3050 cm^{-1}). ^1H NMR showed an aldehyde proton (9.82, CHO) correlated to methylene protons (2.30 and 2.50, 6- CH_2), three methyl groups (0.94, 6H, 2- CH_3 , and 1.06, 3H, 1- CH_3) and methylene protons (2.26 and 2.55, 5-H) correlated to an olefin (5.57, 4-H), which were connected to another olefin methine proton (5.50, 3-H). A long-range correlation between the 5-H and 3-H was observed. NOE was also observed in the 3-H when the signal of 2- CH_3 was irradiated. ^{13}C NMR data, which were obtained in CDCl_3 , showed 10 signals at 22.4 (q), 22.8 (q), 23.2 (q), 44.5 (t), 45.8 (s), 48.9 (s), 51.3 (t), 126.2 (d), 141.6 (d), and 191.9 (d) ppm. These data also supported the structure. Aldehyde 9, which was a new compound, and 10 might be derived from 5. The Kovats indices on DB-Wax and MS spectral data of 3–10 are summarized in Table 2.

The new peaks I, II, and III (Figure 4b) in the photoproducts of lemon flavor were assigned to com-

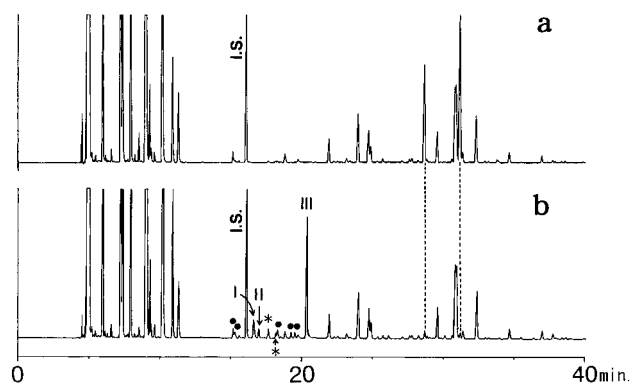


Figure 4. GC chromatogram of a lemon flavor: (a) lemon flavor stored at 30 °C in the dark; (b) lemon flavor irradiated by UV at 30 °C. I.S., internal standard (2-octanol). The new peaks shown by I, II, and III, dots (●), and asterisks (*) appeared in chromatogram b.

pounds **5**, **6**, and **3** in the photoproducts from citral, respectively. Especially **6**, expressing a dusty odor, was a characteristic product in water solution. Peaks indicated by dots (●) in Figure 4b were also assigned to the photoproducts from citral. Peaks designated with asterisks (*) were limonene oxides, which were presumed to be obtained by the oxidation with the rest of oxygen (0.5 ppm). It was suggested that the photolysis of citral should be one of the main factors which allows deterioration of other components forming a lemon odor. The odorous change of lemon flavor was suggested to be mainly caused by the decrease of citral and the formation of an off-odor formed by photoreaction of citral, expressing a dusty odor, which was confirmed using GC-sniffing but not identified.

LITERATURE CITED

- Angelo, D. G.; Francesco, B.; Enrico, P. Analytical characteristics of dewaxed essential oil of lemon. *Essenze Deriv. Agrum.* **1970**, *40*, 143–150.
- Büchi, G.; Wüest, H. The total synthesis of the (+)-furoperar-gones. *J. Am. Chem. Soc.* **1965**, *87*, 1589–1593.
- Cieri, U. R. Characterization of the steam nonvolatile residue of bergamot oil and some other essential oils. *J. Assoc. Off. Anal. Chem.* **1969**, *52*, 719–728.
- Clark, Jr., B. C.; Chamblee, T. S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, 1992; pp 229–285.
- Cookson, R. C.; Hudec, J.; Knight, S. A.; Whitear, B. R. D. The photochemistry of citral. *Tetrahedron* **1963**, *19*, 1995–2007.
- Delepine, M. M. A new form of fenchone oxime; characterization of fenchone in the presence of camphor. *Bull. Soc. Chim. Fr.* **1924**, *35*, 1330–1335.
- Erman, M. B.; Volkova, O. O.; Cherkaev, G. V.; Pribytkova, I. M.; Antipin, M. Yu.; Struchkov, Yu. T.; Mochalin, V. B. Structure and properties of the thermal cyclization products of 1-acetoxy-3,7-dimethyl-1,2,6-octatriene. *Zh. Org. Khim.* **1986**, *22*, 2508–2519.
- Freeburg, E. J.; Mistry, B. S.; Reineccius, G. A.; Scire, J. Stability of citral-containing and citralless lemon oils in flavor emulsions and beverages. *Perfum. Flavor.* **1994**, *19*, 23–32.
- Ifuku, Y.; Maeda, H. Antioxidative components of various citrus essential oils recovered from the waste of the in-line juice extractor. *Nippon Shokuhin Kogyo Gakkaishi* **1978**, *25*, 687–690.
- Kaiser, R.; Lamparsky, D. Natural occurrence of photocitral and some of their derivatives (Constituents of verbena oil, 1st communication). *Helv. Chim. Acta* **1976**, *59*, 1797–1802.
- Kimura, K.; Nishimura, H.; Iwata, I.; Mizutani, J. Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. *J. Agric. Food Chem.* **1983**, *31*, 801–804.
- Liu, X.; Macaulay, E. D. M.; Pickett, J. A. Propheromones that release pheromonal carbonyl compounds in light. *J. Chem. Ecol.* **1984**, *10*, 809–822.
- Piironen, V.; Syväoja, E.-L.; Varo, P.; Salminen, K.; Koivisto-inen, P. Tocopherols and tocotrienols in Finnish foods: vegetables, fruits, and berries. *J. Agric. Food Chem.* **1986**, *34*, 742–746.
- Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797–800.
- Schieberle, P.; Grosch, W. Potent odorants resulting from the peroxidation of lemon oil. *Z. Lebensm. Unters. Forsch* **1989**, *189*, 26–31.
- Shimoda, M.; Hirano, K.; Osajima, Y. Concentration of volatile components in foods with porous polymer column. *Bunseki Kagaku* **1987**, *36*, 792–798.
- Varech, D.; Jacques, J. Conformational analysis in the cyclopentane series. V. Preparation of 2(*R*)-methyl-4(*S*)-*tert*-butylcyclopentanone and 2(*R*)-methyl-5(*S*)-*tert*-butylcyclopentanone. *Bull. Soc. Chim. Fr.* **1969**, 3505–3515.
- Wolff, S.; Barany, F.; Agosta, W. C. Novel photochemical rearrangements of citral and related compounds at elevated temperatures. *J. Am. Chem. Soc.* **1980**, *102*, 2378–2386.
- Ziegler, M.; Brandauer, H.; Ziegler, E.; Ziegler, G. A different aging model for orange oil: deterioration products. *J. Essent. Oil Res.* **1991**, *3*, 209–220.

Received for review February 13, 1996. Revised manuscript received October 21, 1996. Accepted October 29, 1996.*

JF960100H

* Abstract published in *Advance ACS Abstracts*, December 15, 1996.