

mechanism for the formation of lactones in samples irradiated under vacuum, however, in samples irradiated in air, formation of γ -palmitolactone was considerably enhanced (Table III). The effect of oxygen on the increased yield of γ -palmitolactone could be attributed to the formation of oxy free radicals leading to the formation of the stable 5-member ring lactone.

ACKNOWLEDGMENT

This work was supported in part by a contract from the U.S. Army and University of Massachusetts Experiment Station Project no. 433.

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[Received May 22, 1981]

✕ Characterization of Minor Constituents in Commercial Oleic Acid¹

SHERMAN S. LIN², YUKINOBU MURASE³, DAVID B.S. MIN⁴, OLIVER A.L. HSIEH and STEPHEN S. CHANG, Department of Food Science, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

ABSTRACT

Minor constituents were isolated from a mixture of commercial oleic acid manufactured from beef tallow by 10 different companies. Silicic acid was used as an adsorbent to isolate the minor constituents. They were first separated into acidic and nonacidic fractions. Each fraction was then separated into numerous subfractions by stepwise gradient elution liquid chromatography, using silicic acid as the adsorbent. The subfractions which had an adverse effect on the color stability of oleic acid during heating were characterized with functional group analysis, elemental analysis and IR spectrophotometry. The minor constituents amounted to ca. 1.18% of the commercial oleic acid. They were complex mixtures with multiple functional groups. Some of the subfractions contained 2-3 times as much oxygen in the molecule as oleic acid. They had molecular weights ranging from 308 to 830 which are from monomers to trimers of oxidized oleic acid. These minor components contained carbonyl, ester and hydroxyl groups and double bonds. Some of the nonacidic minor constituents may contain amide groups. Most of the acidic subfractions were dark red, viscous liquids and the nonacidic subfractions were dark green or greenish-brown, semisolid substances. A relationship was established that the greater the polarity of the minor constituents, the greater is its adverse effect on the color stability of oleic acid during heating.

INTRODUCTION

Commercial oleic acid has a tendency to develop a dark color during heating. Lin et al. (1) reported that this dis-

coloration is partially due to oxidation and partially due to the presence of minor constituents. The effects of the 2 factors are synergistic and thus accentuate the darkening of the commercial oleic acid when it is heated under air. Removal of the minor constituents from commercial oleic acid by silicic acid treatment drastically improved its color stability. The present paper is an attempt to characterize the chemical nature of the minor constituents.

EXPERIMENTAL PROCEDURES

Material Used

The mixed commercial oleic acid (MCOA) used for this investigation was a composite sample of commercial oleic acid manufactured from beef tallow by 10 companies. Each company submitted an oleic acid sample which was a composite of different grades of oleic acid produced by that company.

Isolation and Fractionation of Minor Constituents from MCOA

The procedure for the isolation and fractionation of minor constituents from MCOA is shown in Figure 1, and is essentially the same as that previously reported (2). The sample (29 kg) was passed through 10 parallel chromatographic columns (4.4 x 55 cm), each packed with 300 g of silicic acid (100 mesh, Mallinckrodt 2847). The silicic acid was purified and activated according to the method of Sahasrabudhe and Chapman (3). The oleic acid that passed through the columns was practically colorless and was considered purified oleic acid (POA). The colored substance originally present in MCOA was adsorbed on the silicic acid column. The silicic acid columns which retained the minor constituents of MCOA were first eluted with hexane to remove all the residual free fatty acids. It was then eluted with ethyl

¹ Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903.

² Present address: Anderson Clayton Foods, PO Box 63, Richardson, TX 75080.

³ Present address: Asahi Denka Kogyo Co., Ltd., 7-1, Higashioku, Arakawa-ku, Tokyo, Japan.

⁴ Present address: Department of Food Science and Nutrition, Ohio State University, Columbus, OH 43210.

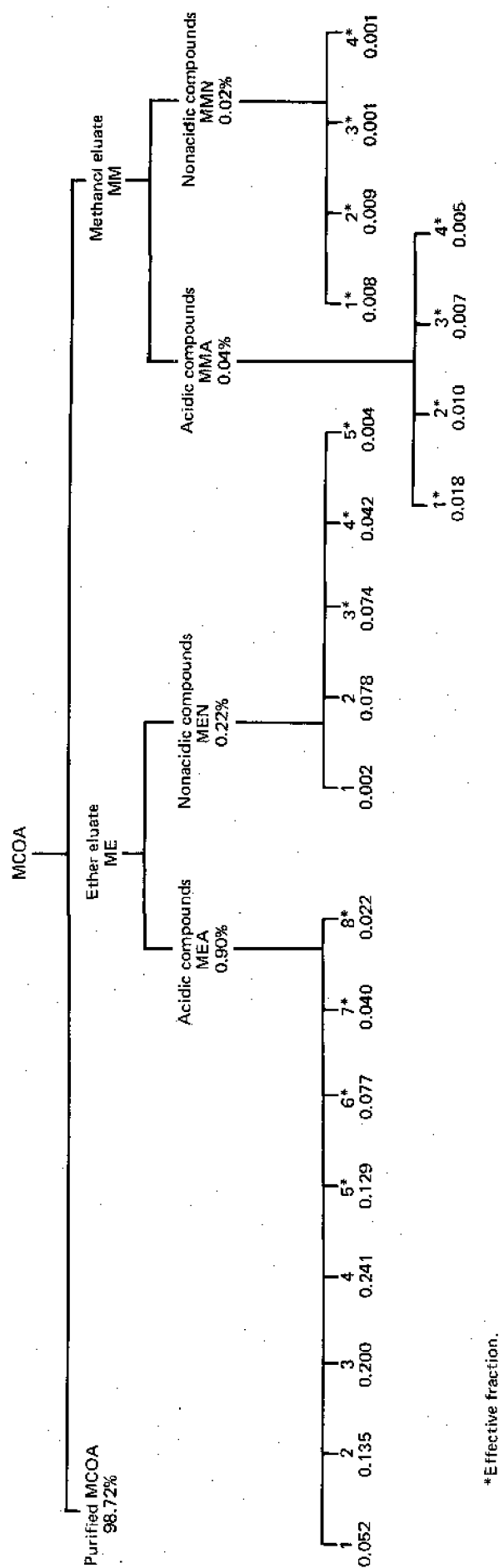


FIG. 1. Isolation and fractionation of minor constituents of mixed commercial oleic acid (MCOA).

ether until no more substance was eluted. The ether eluate (ME) was separated into acid (MEA) and nonacidic fractions (MEN) by extracting its ethyl ether solution with an aqueous 10% sodium carbonate solution. The minor constituents retained on the column after the ether elution were further eluted with an extensive amount of methanol until nothing was eluted with methanol, and the methanol eluate (MM) was also separated into acidic (MMA) and nonacidic (MMN) fractions. The 4 fractions, MEA, MEN, MMA, and MMN of Figure 1 thus obtained, were each fractionated into numerous subfractions with stepwise gradient liquid chromatography by the following solvent system. Fraction MEA solvent system: 5% ether (E) + 95% hexane (H), 10% E + 90% H, 20% E + 80% H, 40% E + 60% H, 60% E + 40% H, 80% E + 20% H, 100% E, and 30% methanol (M) + 70% E; fraction MEN solvent system: 5% E + 95% H, 20% E + 80% H, 40% E + 60% H, 80% E + 20% H, and 100% M; fraction MMA System: 80% E + 20% H, 100% E, 5% M + 95% E, and 10% M + 90% E; fraction MMN: 80% E + 20% H, 100% E, 5% M + 95% E, and 10% M + 90% E.

Effects of Fractions of Minor Constituents on the Color Stability of POA

The method described previously (2) was used.

Analytical Methods

Iodine value and acid value were determined according to AOCS Official Methods (4). Saponification value and hydroxyl value were determined according to Mehlenbacher's procedure (5). Carbonyl value was analyzed by the methods of Bhalerao et al. (6). Molecular weight was determined with a Mechrolab vapor pressure osmometer, Model 301A (Hewlett Packard, Avondale, PA), using methanol as the solvent. Elemental analyses were carried out by the Schwartzkopf Micro Analytical Laboratory, Woodside, NY. IR studies were made with a Beckman IR-8 Spectrophotometer. Color stability measurement was determined according to the AOCS Official Method, Td 2a 64 (4), using a Beckman DG B spectrophotometer.

Hydrogenation and Reduction of Minor Constituents

Hydrogenation was done according to the method of Alexander and Cope (7), using Pd-C as the catalyst. Reduction with NaBH_4 was done according to the method of Chalken and Brown (8). Reduction with LiAlH_4 was accomplished according to the method of Frankel et al. (9).

RESULTS AND DISCUSSION

Material Used

We hoped that representative samples of commercial oleic acid produced by different manufacturers could be used. The Research Committee of the Fatty Acid Producers' Council proposed the use of a blend of equal amounts of commercial oleic acid produced by 10 major manufacturers so that the minor constituents present in different commercial oleic acids could be covered.

Isolation and Fractionation of Minor Components

Activated silicic acid effectively removed the minor components from the MCOA and the eluate from the column was almost colorless. The minor components adsorbed by the silicic acid formed 2 bands: a dark greenish, brown-red on the top, and a yellowish red one beneath it. The total minor components were 1.18% of which 1.12% was ether eluate and 0.06% was methanol eluate.

Effect of Fractions of Minor Constituents on Color Stability

All the subfractions of minor constituents in Figure 1 from the MCOA were tested to study the effectiveness in causing the darkening of purified oleic acid during heating under air. The increases in color, as measured by the Photometric Index at 550 m μ after different fractions of minor constituents were added in various amounts to POA and then heated at 200 C under air for one hour, are shown in Figures 2 and 3.

The degree of darkening of oleic acid during heating was proportional to the amount of minor constituents added. The methanol eluted minor constituents, MMN and MMA, were more detrimental to the color stability of oleic acid than the ether-eluted minor constituents, MEN and MEA. The MEN and MEA are less polar than the MMN and MMA (Fig. 2). Among the subfractions of MMA, the most polar subfraction (MMA-4) gave the most severe adverse effect on the color stability of oleic acid during heating (Fig. 3). These results were the same as those reported by Min et al. (2) in the effects of minor components from tall oil fatty acids (TOFA) on the color stability of purified TOFA during heating. It appears that the more polar the fraction of the minor constituents, the stronger is its effect in causing the darkening of oleic acid during heating.

Characterization of Chromatographic Fractions of the Minor Constituents

Although the fractions of minor constituents in oleic acid were obtained by repeated chromatography, it is evident that they still are not homogenous, pure compounds. The composition of the minor constituents is so complex that obtaining pure compounds is extremely difficult. In addition, even if a few pure compounds are obtained, they may not represent the general chemical nature of the complex mixture. Therefore, selected fractions of the minor constituents were analyzed for their general chemical functional properties, including molecular weight, molecular formula, and the numbers of carboxyl, ester, hydroxyl and carbonyl groups, and double bonds (Table I). The minor constituents had molecular weights ranging from 308 to 830 which correspond to the approximate molecular weights of oxygenated monomers and trimers of oleic acid. These minor constituents were quite polar and rich in oxygen and had hydroxyl, carbonyl and ester groups. They also contained various degrees of unsaturation. The number

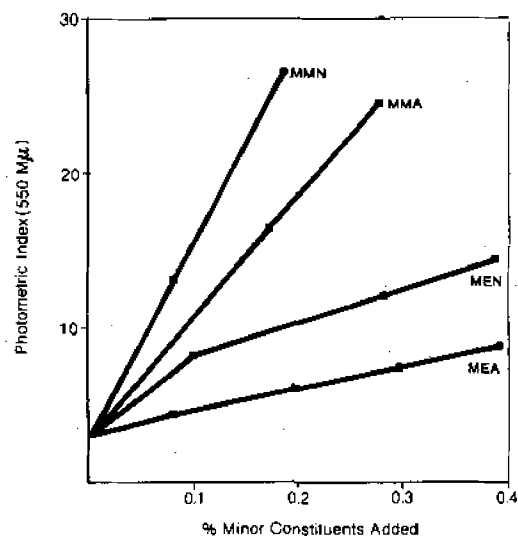


FIG. 2. Effect of minor constituents from MCOA on color stability of oleic acid. MEA = ether-eluted acidic constituents; MEN = ether-eluted nonacidic constituents; MMA = methanol-eluted acidic constituents; MMN = methanol-eluted nonacidic constituents.

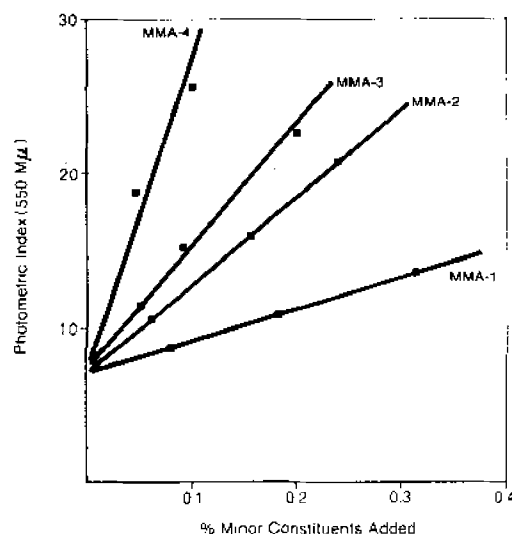


FIG. 3. Effect of subfractions of MMA on the color stability of oleic acid.

TABLE I

Characterization of Minor Constituents of Mixed Commercial Oleic Acid

Fraction	Molecular formula	Molecular weight	-COOH Mol.	-COOR Mol.	-OH Mol.	-C=O Mol.	-C=C- Mol.	Number of oxygen atoms unaccounted for
MEA-5-3	—	370	1.46	0.43	0.61	0.34	0.77	—
MEA-5-4	—	570	1.82	0.94	1.17	0.29	1.13	—
MEA-6-3	—	402	1.30	0.61	1.10	0.31	0.99	—
MEA-6-4	—	574	1.71	1.10	2.09	0.39	2.36	—
MEA-6-5	C ₄₆ H ₇₆ O ₁₃	830	2.49	1.56	2.49	1.83	1.77	0.58
MEA-7	—	540	1.60	0.76	2.62	0.75	1.29	—
MEA-8	—	516	1.45	0.66	1.45	0.51	1.35	—
MEN-3	C ₂₄ H ₄₄ O ₃ N _{0.37}	384	0.00	0.62	0.62	0.25	0.79	—
MEN-5	—	346	0.00	0.39	1.10	0.04	0.79	—
MMA-1	—	348	1.27	0.15	0.68	0.28	1.12	—
MMA-2	C ₂₄ H ₄₁ O ₇	434	1.10	0.68	1.21	0.49	1.06	1.74
MMA-3	—	516	1.32	0.61	1.95	0.38	1.31	—
MMA-4	—	546	1.32	0.64	2.03	0.43	1.41	—
MMN-1	—	318	0.69	1.02	0.27	0.00	1.04	—
MMN-2	C ₁₉ H ₃₆ O ₂ N _{0.7}	308	0.08	0.17	0.74	0.00	0.73	—

of oxygen atoms in the molecule, as determined by elemental analysis, could not be entirely accounted for by the summation of the functional groups analyzed. This might indicate that some oxygen atoms are present in linking the monomeric units or in some unanalyzed functional groups. Generally speaking, as the polarity of the solvent used to elute the fraction from the silicic acid was increased, the hydroxyl groups also increased. For example, MEA-5-3 has 0.61 hydroxyl group/mol and MEA-5-4 has 1.17 hydroxyl group/mol (Table I). This is confirmed by the infrared (IR) spectra of the fractions that the absorption of hydroxyl group at 2.9μ was increased when solvents with increasing polarity were used for elution. Those findings were also observed in the study of minor components in TOFA as reported by Min et al. (2).

Minor components were highly viscous liquids with colors ranging from slight yellow to dark reddish brown or greenish brown. The color and the viscosity of the fractions increased as the polarity of the solvent used for their elution was increased. This was also true in the previous study of TOFA (2).

One interesting observation is that the adverse effect of the fractions of the minor constituents on the darkening of purified oleic acid during heating can be correlated with their polarity and was generally proportional to the amount of hydroxyl groups in their molecules. This is true for both the acidic and nonacidic fractions (Table I, Figs. 2 and 3). The IR spectra of an acidic and a nonacidic minor constituent are shown in Figures 4 and 5. The IR spectra of the nonacidic minor constituent indicated that it might be an amide. However, the N-H and O-H stretching could be overlapped around 2.8 to 3.0μ , and C=O stretching absorptions from amide, aldehyde, ester and other carbonyl groups could overlap one another in the region from 5.7 to 6.4μ . However, elemental analysis did show that nonacidic fractions contain nitrogen from 1.2% for MEN-3 to 3.5% for MMN-2.

Nonaka (10) reported that oxidation of fish oil caused no discoloration, but when certain volatile basic N compounds were reacted with oil, the oil became dark red. Nonaka (11) also observed that fish oil was discolored when it was in contact with commercial casein, but not with purified casein. The color of fish oil in contact with commercial casein apparently was caused by free NH_3 liberated by the decomposition of ammonium salts. Nonaka (12) further reported that some amino acids and amines could cause the same discoloration of fish oil.

The mechanism for an amide functioning as a catalyst for darkening of oleic acid could be manifested in 2 ways. a primary amide could be decomposed to form ammonia and a secondary amide could form an amine. Both ammonia and amines have caused discoloration of heated fish oil (11,12).

Reduction Studies of a Fraction of the Minor Constituents

In order to elucidate the chemical nature of the effective functional groups of the minor constituents in causing the darkness of purified oleic acid during heating, a subfraction, MEA-7, was reduced by the following methods (a) catalytic hydrogenation with Pd-C and H_2 ; (b) with NaBH_4 for 24 hr, (c) with LiAlH_4 for 4 hr, (d) with LiAlH_4 for 36 hr. The effect of the reduced samples on the color stability of purified oleic acid is shown in Figure 6. The results indicated that reduction of MEA-7 with Pd-C and H_2 or NaBH_4 did not affect the activity of MEA-7 significantly, although there was a slight decrease in activity in the second sample. However, the treatment with LiAlH_4 for 36 hr considerably decreased the activity of MEA-7.

A comparison of the IR spectrum of MEA-7 and its

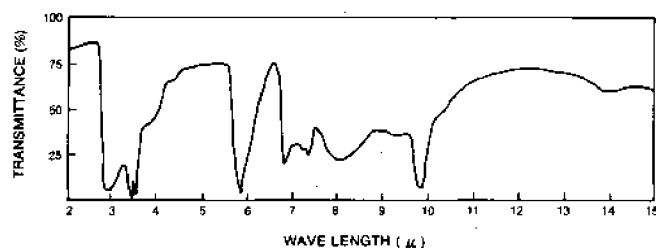


FIG. 4. Infrared spectrum of an acidic fraction of minor constituents isolated from commercial oleic acid.

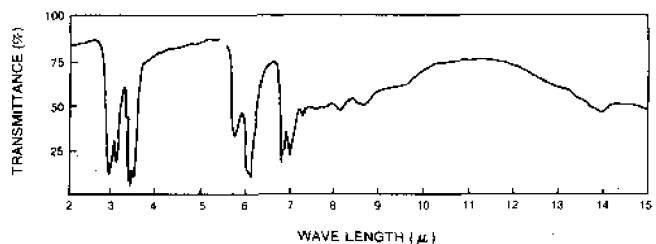


FIG. 5. Infrared spectrum of a nonacidic fraction of minor constituents isolated from commercial oleic acid.

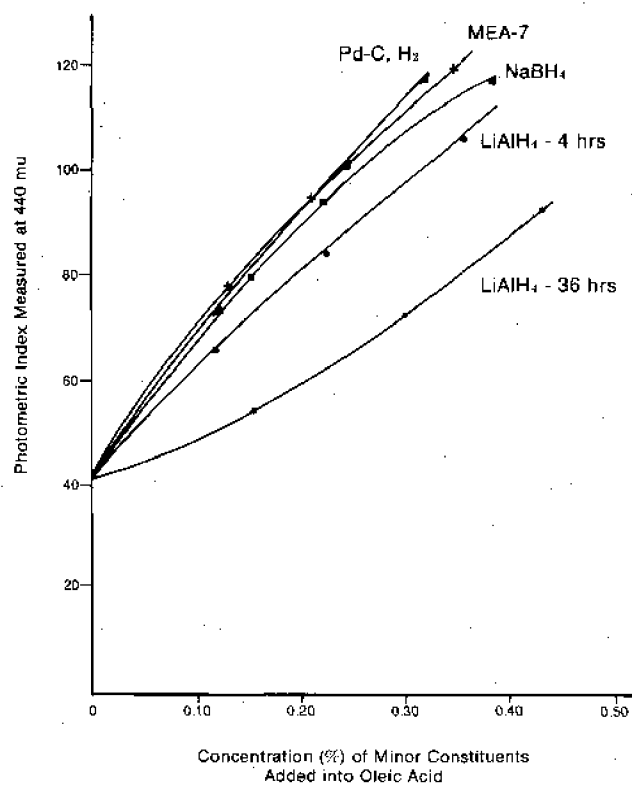


FIG. 6. Effect of MEA-7 and its reduced products on the color stability of pure oleic acid.

reduced forms indicated that catalytic hydrogenation with Pd-C and reduction with NaBH_4 increased the hydroxyl group content of the sample (Fig. 7). This was confirmed by an increase in intensity of the OH stretching vibration band at 2.9μ and also by the appearance of an additional OH bending vibration at 9.2μ for the latter. The increase in hydroxyl group due to the reduction of the unknown functional group(s) by either Pd-C + H_2 or NaBH_4 did not significantly decrease the activity of MEA-7, which suggests that the unknown functional group(s) which was reduced by either Pd-C + H_2 or NaBH_4 is probably of no great im-

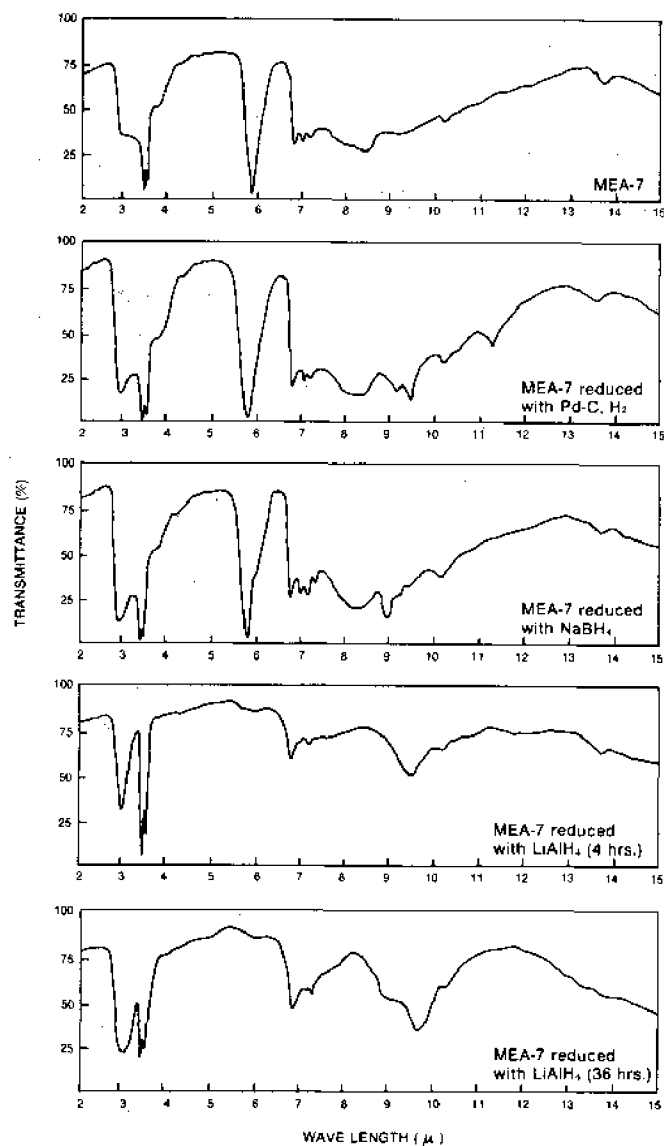


FIG. 7. The infrared spectra of a fraction of the minor constituents before and after various methods of reduction.

portance to the activity of the minor constituents on the color stability of oleic acid.

The LiAlH_4 treatment for 4 hr completely reduced the carbonyl, carboxyl and ester groups of MEA-7 into hydroxyl group as was evidenced by the total disappearance of C=O stretching vibration at 5.8μ and the great increase in OH absorption at 2.9μ of IR spectra (Figs. 4 and 7). Since the LiAlH_4 treatment for 4 hr did not significantly decrease the activity of MEA 7 on the color stability, the C=O groups were probably not responsible for the activity of the minor constituents on the color stability of oleic acid during heating as seen in Figure 6. The comparison between the IR spectra of MEA 7 reduced with LiAlH_4

treatment for 4 and 36 hr in Figure 7 indicated that the intensity of OH absorption at 2.9μ of the 36-hr sample was much greater than that of the 4 hr sample. Since the C=O groups of MEA-7 were completely reduced into OH group after LiAlH_4 treatment for only 4 hr (Fig. 7), the additional increase in OH absorption intensity in the 36 hr sample compared to that of the 4 hr sample is most likely due to the reduction of the unknown functional group(s) other than C=O groups by LiAlH_4 . Since the 36-hr sample reduced the MEA 7 activity significantly, the unknown functional group(s) which can be reduced by LiAlH_4 are highly responsible for the darkening of oleic acid during heating. These facts were also observed in minor components in TOFA as reported by Min et al. (2).

It has been concluded before in this paper that there is a positive correlation between the content of hydroxyl groups in the minor constituents and the adverse effects on the color stability of oil during heating (Table I, Figs. 2 and 3). However, a significant increase in hydroxyl groups by reduction with LiAlH_4 or NaBH_4 resulted in a loss of the effectiveness in causing discoloration; thus, it appears that not all hydroxyl groups are responsible for the darkening effect to purified oleic acid. Min et al. (2) have demonstrated that, in similar cases of TOFA, only the hydroxyl group located in the α position to the double bond in the molecule could cause darkening of the PTOA during heating.

ACKNOWLEDGMENTS

This study was supported by a research fellowship from the Fatty Acid Producers' Council and a research fellowship from the Eastern Regional Research Center, a division of the U.S. Department of Agriculture.

This study was performed as part of NJAES Project No. 10503, supported by the New Jersey Agricultural Experiment Station.

We acknowledge the support and encouragement of A. Benedict Doran.

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(Received October 24, 1980)