Origin of Minor Constituents of Commercial Oleic Acid[1]

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ABSTRACT

Fatty acids were prepared from beef tallow in the laboratory by alkalization and acidification under a nitrogen atmosphere at temperatures below 100 C. The minor constituents in the beef tallow fatty acids were isolated by the silicic acid method as described previously. They were fractionated by silicic acid liquid column chromatography and characterized by chemical functional group analyses, elemental analyses and infrared spectrophotometric methods. The results suggest that the minor constituents in commercial fatty acids were partly present in the original raw material of beef tallow and were partly formed as artifacts during the manufacturing process.

INTRODUCTION

We reported in our previous papers (1-3) that commercial oleic acid contains minor constituents which can be isolated by a silicic acid column method. The minor constituents have been fractionated by gradient elution liquid column chromatography and the fractions characterized. It was found that the minor constituents had an adverse effect on the color stability of oleic acid. When added to purified oleic acid in trace amounts, they could increase the darkening of the oleic acid during heating. This paper is an attempt to trace the origin of such minor constituents in commercial oleic acid.

EXPERIMENTAL PROCEDURES

Materials

The mixed beef tallow (MBT) used was a composite sample of equal weights of beef tallow supplied by each of 10 companies. At the same time, each of these 10 companies also submitted an oleic acid produced from the same batch of beef tallow. The mixed commercial oleic acid (MCOA) was obtained by mixing equal weights of the 10 commercial oleic acids. The study on the characterization of minor constituents in MCOA has been reported (2).

Isolation and Fractionation of Minor Constituents from MBT

The MBT was converted into free fatty acids by alkalization and acidification under a nitrogen atmosphere at a temperature below 100 C (Fig. 1). The crude free fatty acids (23.3 kg) were dissolved in an equal volume of hexane, and the solution was passed through 5 silicic acid columns (4.4 × 55 cm) to yield the purified fatty acids, which were further separated into liquid and solid fatty acids by low temperature solvent crystallization. The minor constituents retained on the column were first eluted with ethyl ether. The eluate (E) was separated into an ether-soluble fraction (ES) and a precipitate fraction (EP) by holding the solution at 0 C for 48 hr. The ether-soluble fraction was then separated into acidic compounds (ESA) and nonacidic compounds (ESN) (Fig. 1). The minor constituents retained on the column after the ether elution were further eluted with methanol, and the methanol eluate (M) was also separated into acidic (MA) and nonacidic (MN) compounds. The 4 fractions thus obtained, viz., ESA, ESN, MA and MN, were fractionated into subfractions by liquid column chromatography as described previously (2,3).

Effect of Fractions on the Color Stability of POA

The method described previously (3) was used. A subfraction of minor constituents was arbitrarily regarded as effective when it caused an increase in color intensity of 20 units/0.1% of added subfractions into purified oleic acid (POA) (1) when measured spectrometrically at 440 mμ after heating at 200 C under air for 1 hr.

Analytical Methods

Iodine value and acid value were determined according to AOCS Official Methods Tg 1a-64 and Te 1a-64, respectively (4). Saponification value and hydroxyl value were determined according to the methods described by Mehlenbacher (5). Carbonyl value was analyzed by the method 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 done by Schwartzkopf Micro Analytical Laboratory, Woodside, NY. Infrared (IR) studies were made with a Beckman IR-8 spectrophotometer. Color stability measurement was determined according to AOCS Official Method Td 2a-64 (4), using a Beckman DB-G spectrophotometer.

RESULTS AND DISCUSSION

Effect of Fractions of Minor Constituents on Color Stability

All the fractions of minor constituents from MBT in Figure 1 were tested for their effectiveness in darkening of POA during heating under air. The darkening effect of the subfraction of ESA and ESN of minor constituents from MBT are shown in Figures 2 and 3. The degree of darkening of POA during heating was proportional to the amount of minor constituents added. The effects of minor constituents from MBT were very similar to those of the minor constituents isolated from the MCOA (2) with respect to causing the darkening of POA.

One observation is that the effectiveness of the fractions of the minor constituents on the darkening of purified oleic acid during heating was well 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, Fig. 2 and 3). This observation is further supported by the size of the IR absorption peak of the hydroxyl group at 2.9 μ as shown in Figures 4 and 5.

The elemental analysis of MBT subfractions ESN-4 and MN-4 contained 2.07 and 2.66% of nitrogen, respectively

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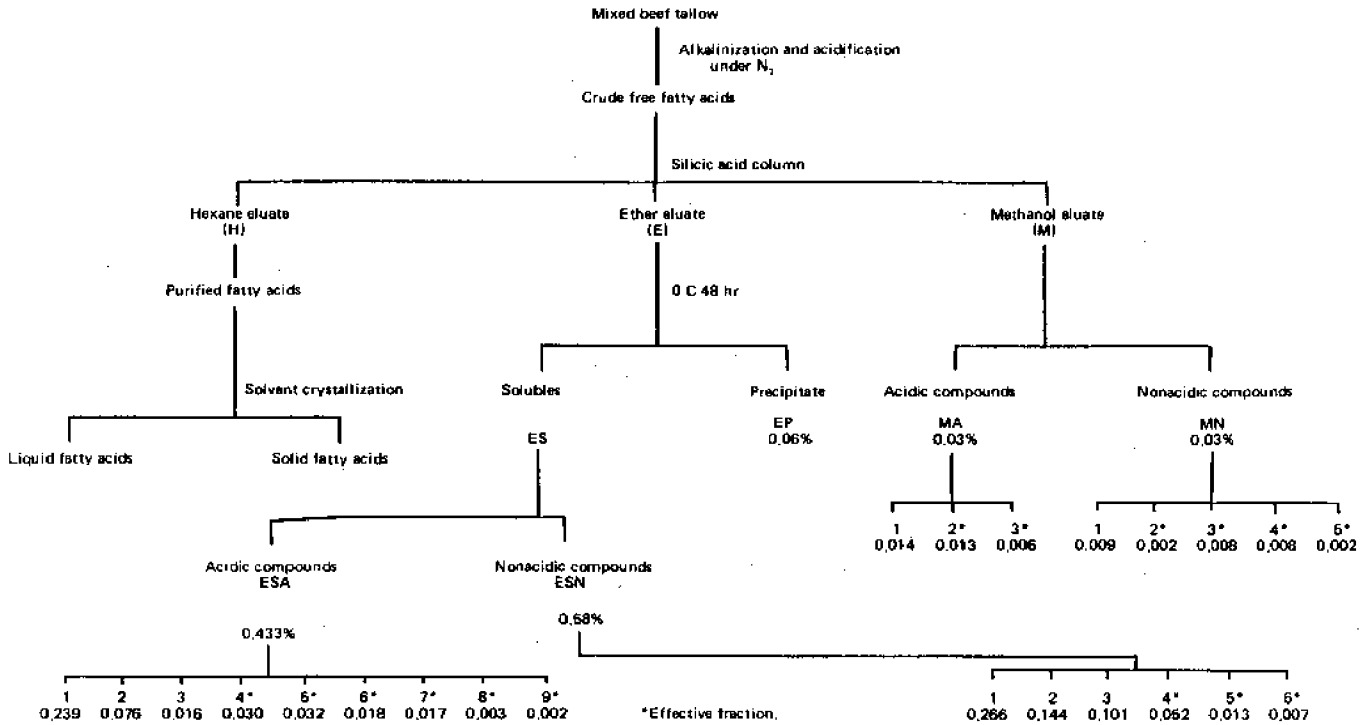

FIG. 1. Isolation and fractionation of minor constituents of mixed beef tallow (MBT).

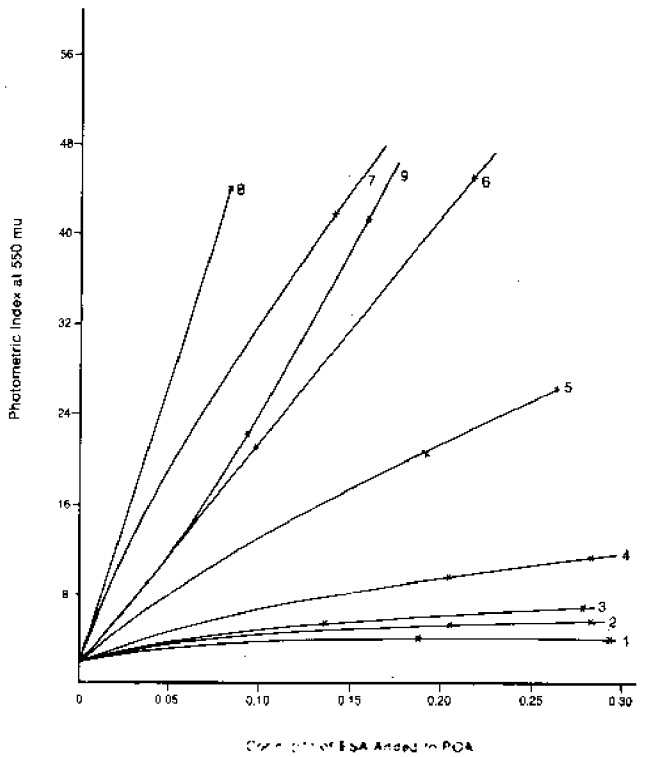

FIG. 2. Effect of subfractions of ether-eluted acidic minor constituents (ESA) on color stability of purified oleic acid.

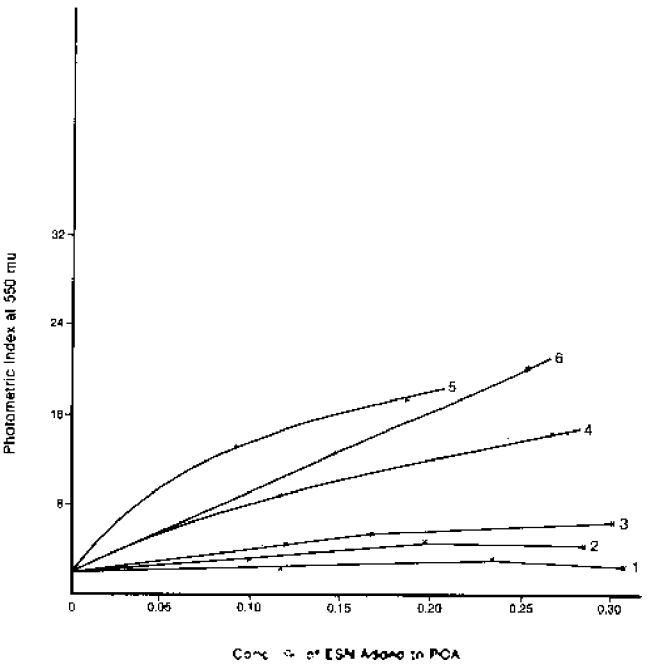

FIG. 3. Effect of subfractions of ether-eluted nonacidic minor constituents on color stability of oleic acid.

TABLE I

Characterization of the Minor Constituents from Mixed Beef Tallow

Fraction	Molecular formula	Molecular weight	-COOH/ mol	-COOR/ mol	-OH/ mol	-C=O/ mol	-C=C-/ mol	Number of oxygen atoms unaccounted for
ESA-4	–	520	1.66	0.17	0.56	0.63	1.07	–
ESA-5	–	712	2.07	0.66	3.16	0.70	1.45	–
ESA-6	–	786	2.02	0.73	3.12	0.94	1.74	–
ESA-7	$C_{59}H_{96}O_{16}$	1062	2.32	0.95	2.59	1.80	2.38	5.09
ESA-8	–	850	2.09	1.09	4.68	0.56	1.55	–
ESA-9	–	424	0.99	0.22	3.90	0.08	–	–
ESN-4	$C_{20}H_{38}O_{3.2}N_{0.3}$	384	0.00	0.68	1.27	0.16	0.78	–
ESN-5	–	294	0.00	0.41	1.03	0.12	0.54	–
MA-2	$C_{36}H_{58}O_9$	646	1.38	0.33	1.28	0.47	0.92	3.83
MA-3	$C_{33}H_{58}O_{11}$	634	1.35	0.00	2.41	0.23	0.25	5.66
MN-2	–	318	0.00	0.67	2.07	0.03	0.59	–
MN-3	–	360	0.00	0.35	1.06	0.07	0.53	–
MN-4	$C_{23}H_{44}O_4N_{0.75}$	402	0.00	0.62	1.10	0.00	0.59	–
MN-5	–	484	0.00	0.59	1.92	0.00	0.61	–

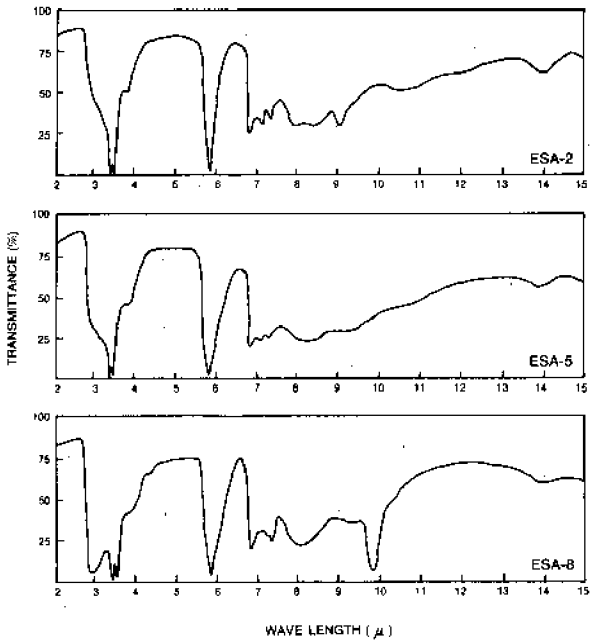

FIG. 4. Infrared spectra of acidic fraction of minor constituents isolated from fatty acids prepared in the laboratory from beef tallow.

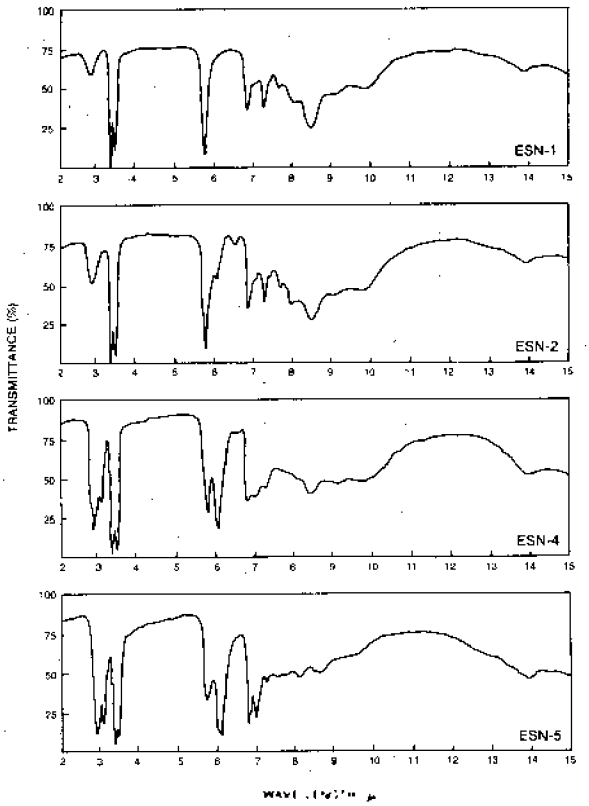

FIG. 5. Infrared spectra of nonacidic fractions in fatty acids prepared in the laboratory from beef tallow.

(Table I). The IR spectra of these compounds suggested the possible presence of amide groups. Detailed IR spectra interpretation on amide and the possible effects of amide on the color stability of oleic acid during heating are discussed in detail in a previous paper (2).

Chemical Nature of the Minor Constituents

The isolated minor constituents were highly viscous liquids with colors ranging from slight yellow to dark reddish-brown. The analyses of selected fractions of the minor constituents from beef tallow fatty acids are shown in Table I. The results indicate that the minor constituents were highly oxygenated compounds containing hydroxyl and carbonly groups and had various degrees of unsaturation. The number of oxygen atoms in the molecule as determined by elemental analysis could not be entirely accounted for by all the functional groups analyzed. This might indicate that some oxygen atoms were present in linking the monomeric units, or in some unanalyzed functional groups.

The chemical analyses of the minor constituents of fatty acids of beef tallow prepared in the laboratory (Table I) indicated that they are the same type of compounds as those found in MCOA (2). However, there are a number of significant differences. The molecular weights of the minor constituents isolated from MBT ranged from 318 to 1062, which is considerably higher than those of the minor constituents of MCOA, which ranged from 308 to 830 (2). None of the minor constituents (except ESA 8) isolated from the MBT contained more than one ester group. This was different from the minor constituents isolated from the MCOA which had 3 fractions containing probably 2 ester groups (2). The minor constituents isolated from the MBT contained a significantly higher amount of hydroxyl groups than those isolated from the MCOA. Some of the minor constituents isolated from the MBT contained as many as 5 hydroxyl groups per molecule, whereas those from the MCOA contained no more than 3.

TABLE II

Comparison of the Quantity of Minor Constituents from Mixed Commercial Oleic Acid and Mixed Beef Tallow (%)

	Total minor constituents	Effective minor constituents				Total effective minor constituents
		EA	EN	MA	MN	
Mixed commercial oleic acid	1.18	0.27	0.12	0.04	0.02	0.45
Mixed beef tallow	1.09	0.10	0.07	0.02	0.02	0.21

Origin of the Minor Constituents in Commercial Oleic Acid

Similar minor constituents with similar chemical functional groups and similar effects on the color stability of purified oleic acid during heating were found in both commercial oleic acid (2) and in fatty acids produced from beef tallow in the laboratory (Table I and Fig. 2-5). Since the fatty acids were prepared from beef tallow in the laboratory under an inert atmosphere at temperatures no higher than 100 C, it is reasonable to assume that a portion of the minor constituents in the commercial oleic acid was originally present in the raw material, beef tallow.

The comparison of the amount of the minor constituents isolated from the MCOA and the MBT is shown in Table II. There was a total of 1.18% minor constituents in the MCOA, and only 1.09% in the MBT. Furthermore, the total amount of the minor constituents which could effect the darkening of oleic acid during heating was 0.45% in the MCOA and only 0.21% in the MBT. This indicated that the minor constituents in the MCOA were not entirely present in the raw material, the MBT. They might be partly produced during the manufacturing process.

ACKNOWLEDGMENTS

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Qualitative and Quantitative Comparison of Minor Constituents in Different Commercial Oleic Acids[1]

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ABSTRACT

The minor constituents from a high quality commercial oleic acid (SCOA) were isolated and fractionated by liquid column chromatography. They were chemically characterized and their effects on the color stability of oleic acid during heating were determined. The results were compared qualitatively and quantitatively with those from a mixture of commercial oleic acids (MCOA) manufactured by 10 companies. It was found that, qualitatively, SCOA and MCOA contained the same type of minor constituents. However, quantitatively, the MCOA contained 1.18% of minor constituents whereas the SCOA contained only 0.81% of minor constituents. The amount of effective minor constituents which had an adverse effect on the color stability of oleic acid during heating was 0.09% for SCOA vs 0.45% for MCOA.

INTRODUCTION

We reported in our previous papers (1-3) that commercial oleic acid contains minor constituents which can be isolated by a silicic acid column method. The minor constituents have been fractionated by gradient elution liquid column chromatography and the fractions characterized. It was found that the minor constituents had an adverse effect on the color stability of oleic acid. When added to purified oleic acid in trace amounts, they could increase the darkening of the oleic acid during heating.

This paper reports the qualitative and quantitative comparison between the minor constituents isolated from a single commercial oleic acid of the highest quality and

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