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Studies on the Chemistry of the Fatty Acids VIII The Reaction of Thiocyanogen with Linoleic and Linolenic Acids

The Application of the Thiocyanogen Reaction to the Determination of these Acids in Fatty Acids Mixtures*

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THE reaction of thiocyanogen with the unsaturated fatty acids and their glycerides was proposed by Kaufmann (1, 2, 3, 4) and by Kaufmann and Keller (5) as the basis for the analysis of fatty acid mixtures containing oleic, linoleic and linolenic acids. Simultaneous equations were set up, based on iodine and thiocyanogen numbers, for the calculation of the composition of such mixtures. These equations were derived from the fundamental assumptions that the iodine numbers of the unsaturated acids were equal to the theoretical values and that the thiocyanogen numbers of oleic, linoleic and linolenic acids were values equivalent to reaction with one, one, and two double bonds respectively.

In his study of the reaction of thiocyanogen with linoleic acid, Kaufmann (2) employed a specimen of trilinolein, the iodine number of which was 169.1 (theory 173.3). The thiocyanogen number was 79.5 and 82.5 at 5 and 17 hours reaction time respectively (theory for one double bond, 86.7). In evaluating the reaction of thiocyanogen with linolenic acid Kaufmann and Keller (5) avoided the use of acid prepared by debromination because they were not sure that it was identical with the natural acid. They based their conclusions, therefore, on thiocyanogen values obtained on a mixture of linoleic and linolenic acids obtained from linseed oil acids by a procedure whereby most of the saturated acids and oleic were removed by crystallization of the lithium soaps. The composition of the mixture was the same whether calculated directly from the iodine number as a binary mixture or from simultaneous equations, based on the assumption that the reaction of thiocyanogen was the theoretical for one and two double bonds with linoleic and linolenic acids respectively.

Since Kaufmann's original proposals several investigators, working with debromination linoleic and linolenic acids, have observed variations from the assumed theoretical thiocyanogen numbers. Waterman, Bertram and van Westen (6) reported a thiocyanogen number of 92.5 for linoleic acid (theory 90.6). Riemenschneider and Wheeler (7) in a study of the reaction curve of methyl linoleate reported a value of 89.0 (theory 86.3) for a 24 hour reaction period, and Wheeler, Riemenschneider and Sando (8) in a similar study of trilinolein observed a value of 90.5 (theory 86.7). They advocated a four-hour reaction period for analysis of glyceride mixtures, since the reaction with triolein and trilinolein was almost exactly the theoretical in that period. Kass, Lundberg and Burr (9) found 96.3, and Hilditch and Murti (10) 95.9 for linoleic acid.

Kimura (11) observed that thiocyanogen reacted with debromination linolenic acid to a value between one and two double bonds. Shinowara and Brown (12) found a thiocyanogen number of 161.0 for linolenic acid (theory for two double bonds, 182.5). Very recently Kass, Loeb, Norris and Burr (13) reported 167.3 and Hilditch and Murti, 162.5, for debromination linolenic acid. In these reports in which actual reaction curves were described (7, 8, 13), either with the acids or their esters, it was found that the thiocyanogen reaction was continuous up to and beyond 24 hours with the two and three bond acids and that the reaction did not entirely cease at any point of partial saturation.

In 1936 a committee of The American Chemical Society drew up a procedure of analysis (14). The reagent in this procedure was 0.2 N. with respect to thiocyanogen instead of 0.1 N as proposed by Kaufmann. This procedure was employed by Kass et al (8, 13).

In the more recent reports cited above the possibilities that the debromination acids which were used might contain appreciable amounts of isomeric acids and that these conceivably might react at a different rate from that of the natural acid have been overlooked. Both Kaufmann and Keller (5) and Kimura (11) suggested that the acids might be heterogeneous without, however, the confirmation of experimental evidence. Some light is thrown on this angle of the question by the data of Shinowara and Brown (12) who reported thiocyanogen values of 152.6 and 154.1 on two specimens of linolenic acid of iodine numbers 260 and 262 respectively; these specimens were pre-

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pared directly from natural sources. Calculated to the basis of 100 percent purity this would indicate a value of about 163 for the pure acid. This observation, together with our own value of 95.0 for a specimen of crystallization linoleic acid, previously reported by Brown and Frankel (15), seems to indicate that crystallization and debromination specimens of the two acids do not greatly differ in their reaction with thiocyanogen. Kass, Lundberg and Burr found a thiocyanogen number of 94.7 for linoelaidic acid and Kass, Nichols and Burr (16) a value of 149.7 for elaidolinolenic acid. This last value would appear to contradict the previous citations.

When the present work was begun over two years ago we desired to study the thiocyanogen reaction with linoleic and linolenic acids of the very highest purity. One of our first observations was that our debromination linoleic and linolenic acids, although pure as evaluated from the iodine number, were separable into fractions by low-temperature crystallization. The present report was delayed for a number of reasons, but especially by the necessity of studying this problem of purifying the debromination acids. As a result (17) we have shown that our specimens of debromination linoleic and linolenic acids contained approximately 12 and 15 percent respectively of contaminating isomeric acids which probably were the result of isomerization during debromination. As a result of repeated crystallization, we have had available specimens of the two acids which are practically free from the contaminating isomers.

In the present work we have studied the rate of decomposition of the thiocyanogen reagent and have suggested certain modifications in both preparation and composition of the reagent. The reaction rates of thiocyanogen with oleic, linoleic and linolenic acids with varying excess of reagent and at three different temperatures have been studied. Thiocyanogen values for linoleic and linolenic acids have been determined on a number of different specimens of these acids and these values compared with those by the Kaufmann and official procedures. Several synthetic mixtures of fatty acids of known composition have been prepared and their composition satisfactorily calculated from equations based on our data.

Experimental

Preparation of Thiocyanogen Reagent

The procedure employed in making up the thiocyanogen reagent is essentially the same as that described in the official method (14) with certain modifications. Anhydrous acetic acid was prepared by refluxing glacial acetic acid with 11 percent its volume of acetic anhydride. Fifty grams of dry lead thiocyanate was suspended in 900 cc. of the anhydrous acid and to this was slowly added a solution of 20 grams of bromine in 100 cc. of carbon tetrachloride, so that at no time was a large excess of bromine present. After the addition of bromine was complete the solution was filtered. The resulting solution is somewhat stronger than 0.2 N. After titration, the thiocyanogen solution was diluted with sufficient anhydrous acid containing 10 percent carbon tetrachloride to a concentration of 0.2 N. The added carbon tetrachloride prevents hard freezing under storage conditions, increases the solvent power of the reagent and somewhat improves titration conditions by holding the thiocyanated specimen in solution during the titration. Our data show no indication that the presence of the tetrachloride materially affects the rate of reaction.

Kass et al (13) and we have observed that the reagent when prepared by the official method is usually weaker than 0.2 N. The 0.2 N solution is to be preferred over the 0.1 N solution of Kaufmann because of the smaller volumes of reagent required in the analysis of high thiocyanogen number mixtures, more especially, when a 60-75 percent excess of reagent is required under the conditions of the analysis.

Analytical Procedures

Twenty-five cc. portions of the reagent, stored at 3°C., are transferred to dry 125 cc. or 200 cc. glass stoppered containers and these are placed in a thermostatically controlled compartment maintained at 16° ± 0.5°. After the reagent samples have come to this temperature, specimens of acids are weighed into these bottles, the amounts of samples being such as to react with 25-40 percent of the thiocyanogen, thus providing a 60-75 percent excess of reagent. After 24 hours reaction, 2.5 cc. of 50 percent potassium iodide are added with stirring, followed by 25 cc. of water. The solution is titrated with thiosulfate. Blanks are run at the beginning and end of the reaction period in order to note change in the reagent.

We have chosen a 16° reaction temperature instead of the 18-21° of the official method for two reasons. In some laboratories, especially in the winter it may be easier to maintain this as a constant temperature without employing heat, and further, the rate of decomposition of the reagent during the reaction period may be expected to be somewhat less than at the higher temperature. Actually, either temperature is satisfactory, and in fact, the results by the two temperatures agree almost within the experimental error (see later data).

The Rate of Decomposition of the Thiocyanogen Reagent at Various Temperatures

Portions of 0.2 N thiocyanogen reagent, prepared as described above, were allowed to stand in glass stoppered bottles at —22°, 3°, 16°, and 28°C. The —22° solution was partly frozen during the experiment. The solution kept at 3° remained a liquid, although the freezing point of the solution is about 10°. The solutions were adjusted to 16° before sampling. Ten cc. portions were removed and titrated with sodium thiosulfate. The results are given in Table I.

TABLE I

The Rate of Decomposition of Thiocyanogen Reagent

		cc. 0.1 N Thiosulfate			
Days	Temp.	28°	16°	3°	—22°
0.0	....	20.60	20.60	20.60	20.60
1.0	....	20.26	20.50	....	....
2.3	....	19.33	20.50	....	....
3.9	....	16.60	20.42	....	....
5.1	....	10.70	....	20.60	....
6.0	....	6.47	....	....	....
7.1	....	4.09	19.85	....	....
10.3	....	2.06	19.03	....	....
16.1	....	1.52	16.04	20.38	....
24.0	....	....	7.37	20.36	....
36.0	....	....	2.90	20.22	....
56.0	....	....	....	19.38	20.54

At 3° the solution had decomposed only 2 percent in 36 days; at —22°, only 0.3 percent in 56 days. It may be concluded that storage at 3° or lower is a convenient method of stabilizing the reagent. In the routine use of thiocyanogen solutions stored at 3°, we have noted no appreciable change in reactivity up to a period of three weeks.

Reaction of Thiocyanogen with Oleic, Linoleic and Linolenic Acids under Various Conditions

In our first experiments the rates of reaction of thiocyanogen with the several unsaturated acids were followed at three temperatures and with varying excess of reagent.

Most of the specimens of acids used in this and in later experiments have been previously described (17) and specimen numbers refer to those in Table I of the previous report and likewise to those in Table V of this report. In the present experiment the acids used were:

Oleic: iodine number, 89.8; thiocyanogen number, 87.2. By calculation this acid contained 3.2% saturated acids and 3.0% linoleic acid.
Linoleic: No. 2 Table V.
Linolenic: No. 7 Table V.

Samples of the acids were weighed into 50 cc. volumetric flasks. The temperature of the flask and of the thiocyanogen reagent was adjusted to the temperature desired and exactly 48.5 cc. of reagent added to the flask. The contents of the flask were quickly made up to volume with a mixture of anhydrous acetic acid and carbon tetrachloride. In all of these solutions the concentration of carbon tetrachloride was 40 percent since it was desired that even at 3° no crystals of solvent should form which would result in increasing the concentration of thiocyanogen. Series of solutions were set up for each acid with varying excess of reagent and for three temperatures, 3°, 16° and 25°. Five cc. portions were removed from time to time and the unreacted thiocyanogen titrated with thiosulfate. Blanks, run on separate flasks at the three temperatures, showed 0.2 percent, 0.5 percent, and 1.2 percent decomposition respectively for 24 hours. Experimental data and results are described in Table II.

The data in the preceding table show that the reaction of thiocyanogen with the several unsaturated acids is a function of time, temperature and concentration of reagent (excess). Reactions are somewhat slower at the lower temperatures and with lower percent excess of reagent. In Table III we have recorded thiocyanogen numbers for exactly 6, 12, 24, and 48 hours. These values were obtained by interpolation from large scale curves plotted from the preceding data. They are included primarily for reference purposes in that they may be useful, if desired, for setting up revised simultaneous equations based on any of the times noted for estimating these acids in mixtures. It should be observed, however, that the data in Tables II and III are not quite as accurate as those obtained by the use of individual samples for analysis.

The actual 24 hour values at 16° are slightly lower than those described later. The difference may be due to the use of a higher concentration of carbon tetrachloride or to the use of less pure samples of acids. Some discrepancies are to be noted also between individual values in Table II. Nevertheless, the data are sufficiently consistent to demonstrate the effect of the three variables studied. It is to be noted here that the values for oleic acid approach a maximum of 87.5-88.0, which for this specimen is about two units lower than the iodine number. We feel that previous work on oleic acid and later work in this laboratory on a very pure specimen of oleic acid agree on the fact that the iodine and thiocyanogen numbers of pure oleic acid are practically equal to the theoretical for one double bond.

Since the data in Table II were based on titration of 5 cc. portions of reaction mixture, rather than on quantities such as are employed in the official procedure, we have redetermined the reaction rates for linoleic and linolenic acids at 16° under conditions similar to those employed in the analytical procedure previously described. The reagent was 0.2 N and contained 10 percent carbon tetrachloride. The linoleic acid was a specimen which had been crystallized 12 times from petroleum ether, No. 5; iodine No. 181.0; molecular weight, 280.9. The linolenic acid was a 6-times crystallized debromination acid, No. 13, iodine No. 272.9; molecular weight, 278.0. Samples were weighed out into small glass cups and dropped into 25 cc. portions of reagent, previously cooled to 16°. The samples were of such size as to afford an approximately 66 percent excess of reagent at 24 hours. Blanks showed 0.2 percent and 0.46 percent decomposition at 24 and 48 hours

TABLE II

Reaction of Thiocyanogen with Unsaturated Acids

Oleic Acid

Time Hours 25°	SCN No. 74%*	Time Hours 25°	SCN No. 45%*	Time Hours 16°	SCN No. 75%*	Time Hours 3°	SCN No. 37%*	Time Hours 3°	SCN No. 79%*	Time Hours	SCN No.
0.35	73.0	0.30	63.8	2.62	85.1	1.18	77.5	1.18	84.2	....	....
1.67	85.5	1.65	84.9	5.47	86.8	4.37	85.2	4.37	86.3	....	....
4.40	87.6	4.35	86.6	15.2	87.4	7.77	87.0	7.60	86.9	....	....
13.2	87.9	13.1	87.8	49.0	88.7	17.8	87.2	17.7	87.2	....	....
24.9	87.9	24.9	88.6	....	....	166.7	87.8	166.7	87.2	....	....

Linoleic Acid

25°	60%*	25°	81%*	16°	46%*	16°	76%*	3°	41%*	3°	79%*
0.40	69.3	0.45	77.0	1.93	86.3	1.93	89.3	0.87	61.3	0.87	68.3
1.67	89.9	1.70	90.1	5.80	90.2	5.85	90.7	2.45	81.9	2.43	88.0
4.45	92.3	4.50	92.6	15.5	92.1	15.5	91.8	6.68	89.9	6.68	91.9
13.2	94.1	13.2	94.4	28.7	93.9	28.7	94.5	16.8	91.2	16.8	92.6
24.9	96.0	25.0	96.8	....	....	....	....	48.3	92.5	48.3	94.6
....	....	....	....	....	....	....	....	166.7	96.3	166.7	99.5

Linolenic Acid

25°	47%*	25°	77%*	16°	45%*	16°	74%*	3°	39%*	3°	77%*
0.48	87.6	0.52	100.5	1.27	108.8	1.27	114.4	0.87	76.0	0.87	85.6
1.97	130.1	2.02	137.4	5.70	144.2	5.72	152.2	2.45	107.9	2.43	120.9
6.30	153.6	6.37	157.1	15.4	157.2	15.4	160.5	6.67	133.0	6.67	148.4
12.3	161.1	12.4	162.7	28.9	162.9	28.8	167.2	16.9	149.8	16.9	160.5
23.8	165.6	23.8	167.7	49.2	166.6	49.2	168.7	28.8	156.8	28.8	164.0
....	....	....	....	....	....	....	....	166.7	167.8	166.7	174.1

* These values are % of SCN remaining at 24 hours, based on the amount of original SCN.

TABLE III

The Effect of Variations in Time of Reaction, Temperature and Excess of Reagent upon Thiocyanogen Values

Fatty Acid	°C.	Excess $(SCN)_2$-%	Thiocyanogen No. at 6 hr.	12 hr.	24 hr.	48 hr.
Oleic	3	37	86.0	87.0	87.5	87.5
	3	79	86.5	87.0	87.5	88.0
	16	75	87.0	87.5	88.0	88.0
	25	74	87.5	88.0	88.0	
Linoleic	3	41	89.5	90.5	91.5	92.5
	3	79	91.5	92.0	93.0	94.5
	16	46	90.0	91.0	93.5	
	16	76	90.5	91.5	94.0	
	25	60	93.0	94.0	96.0	
	25	81	93.0	94.5	97.0	
Linolenic	3	39	130.5	144.0	154.5	160.5
	3	77	146.0	157.0	163.0	166.5
	16	45	145.0	155.0	161.5	166.5
	16	74	152.5	159.5	165.0	168.5
	25	47	153.0	160.5	166.0	
	25	77	156.5	162.5	168.0	

respectively. Analyses were completed at various time intervals. The detailed results are shown in Table IV and the reaction curves in Figure 1.

From the curves in Fig. 1 it is apparent that with both acids the reaction with thiocyanogen may be roughly divided into a period of rapid reaction up to about 2 and 9 hours respectively for linoleic and linolenic acids and into a period of slow steady increase beyond these times. From these data it would be possible to set up corrected simultaneous equations for use in analysis for any time after nine hours. For mixtures containing only oleic and linoleic acids any time after 2-3 hours could be chosen. The 24 hours values in this experiment were 97.2 and 166.5 for linoleic and linolenic acids respectively.

Determination of Thiocyanogen Values under Various Conditions of Analysis

Thiocyanogen values have been determined under various conditions and on a number of specimens of linoleic and linolenic acids. Most of the details of the various determinations will be obvious from the data in Table V, which follows. In further explanation it should be said that results by the modified procedure refer to the use of the official American Oil Chemists' procedure, with the modifications noted above, namely, a 0.2 N reagent containing 10 percent carbon tetrachloride and a reaction temperature of 16 ± 0.5°. The Kaufmann solution is 0.1 N with respect to thiocyanogen and contains 8 percent acetic anhydride and 25 percent carbon tetrachloride. Two special solutions noted in the table were made up to contain 10 percent carbon tetrachloride, but in addition they contained phosphoric acid and pyridinium hydrogen sulfate in 0.2 M concentration. They are included merely because they appeared to increase the reactivity of the thiocyanogen reagent.

TABLE IV

The Rate of Absorption of Thiocyanogen by Linoleic and Linolenic Acids at 16°C.

	Linoleic Acid			Linolenic Acid		
Time Hr.	Sample Wt. g.	$(SCN)_2$ Absorbed*	$(SCN)_2$ No.	Sample Wt. g.	$(SCN)_2$ Absorbed*	$(SCN)_2$ No.
0.33	0.2281	14.14	81.0	0.1322	10.14	100.0
	0.2278	14.17	81.2	0.1317	10.21	101.2
1.00	0.2279	15.85	90.8	0.1319	13.14	130.0
	0.2284	15.90	90.9	0.1314	13.08	129.9
3.00	0.2271	15.95	91.7	0.1315	15.29	151.7
	0.2276	16.05	92.1	0.1318	15.24	150.9
9.00	0.2285	16.43	93.9	0.1324	16.33	161.0
	0.2276	16.35	93.6	0.1318	16.22	160.6
24.0	0.2274	16.92	97.2	0.1321	16.89	166.9
	0.2275	16.94	97.3	0.1319	16.79	166.1
48.0	0.2285	17.70	101.2	0.1316	17.23	171.0
				0.1325	17.35	170.9

* Expressed as ml. 0.1028 N $Na_2S_2O_3$.

Excepting the data obtained with the phosphoric acid and pyridinium sulfate reagents, the results in Table IV show thiocyanogen values from 95.1 to 97.2 for linoleic acid and 162.7 to 167.4 for linolenic acid. These differences are due to variations in procedure, reagent and specimen of acid. The selection and adoption of a standard thiocyanogen number for these acids must, therefore, be quite arbitrary. We have attempted to set up such a standard as follows:

Thiocyanogen values for linoleic acid were taken on the twelve-times crystallized acid with five different thiocyanogen solutions; each value is the result of two or more determinations, by the modified procedure with 60-75 percent excess reagent at 16°. The five values used were 96.1, 96.3, 96.4, 97.0, and 97.2. Average 96.6. This value for linoleic acid is slightly lower than the value by the official method (97.0) and is slightly higher than that by the Kaufman procedure (95.0).

THE REACTION OF THIOCYANOGEN WITH LINOLEIC AND LINOLENIC ACIDS

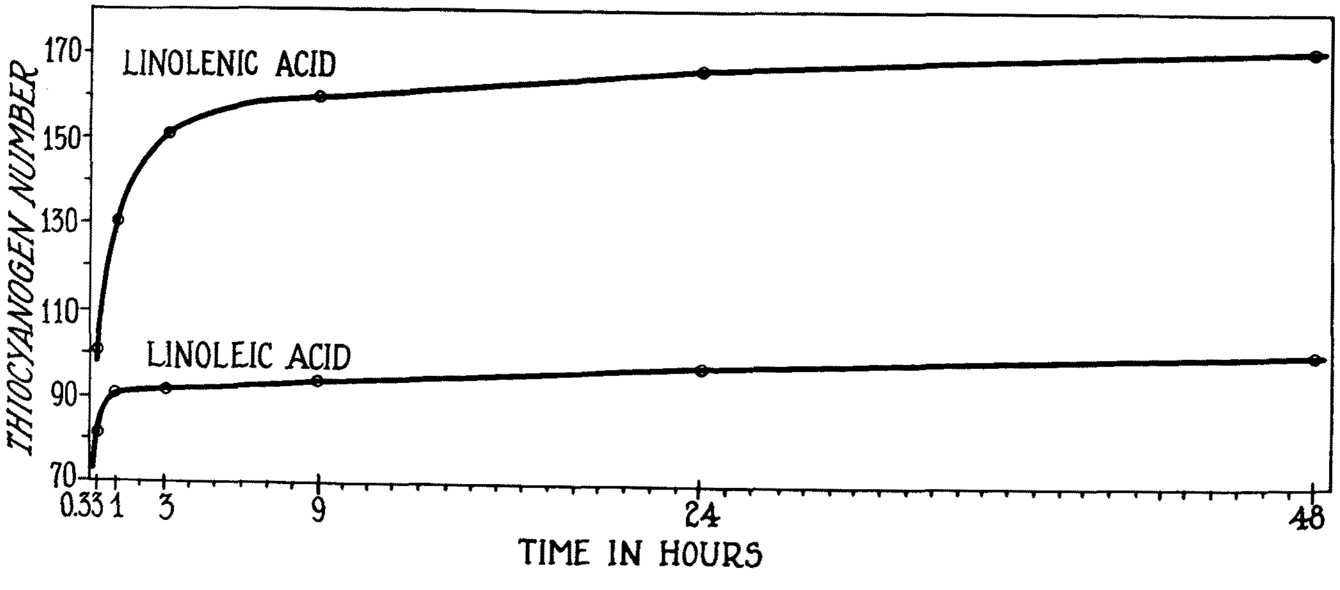

TABLE V

Thiocyanogen Numbers of Linoleic and Linolenic Acids Under Various Conditions

Det'n. No.	Description of Acid	Specimen No.*	Iodine No.	SCN Reagent	Concn. SCN No.	°C.	Excess SCN %	SCN No.
	Linoleic Acid							
1	Debromination	(1)	180.7	Official	0.165	20-22	51	97.0
2	Debromination twice cryst.	(2)	180.9	Kaufmann	0.100	20	60	95.0
3	Debromination twice cryst.	(2)	180.9	Modified	0.204	16	62	95.1
4	Debromination twelve times cryst.	(5)	181.0	Modified	0.204	16	60	96.7
5	Debromination twelve times cryst.	(5)	181.0	Modified	0.204	16	75	97.2
6	Debromination twelve times cryst.	(5)	181.0	Modified	0.206	16	66	97.2
7	Debromination twelve times cryst.	(5)	181.0	Modified H_3PO_4	0.22	16	61	117.8
8	Debromination twelve times cryst.	(5)	181.0	Modified Pyr. SO_4	0.197	16	55	123.0
	Linolenic Acid							
9	Debromination	(6)	272.6	Official	0.185	20-23	61	166.7
10	Debromination four times cryst.	(7)	272.3	Kaufmann	0.100	20	71	165.8
11	Debromination four times cryst.	(7)	272.3	Modified	0.204	16	63	162.7
12	Debromination six times cryst.	(13)	272.9	Modified	0.205	16	60	167.3
13	Debromination six times cryst.	(13)	272.9	Modified	0.205	16	66	167.0
14	Debromination six times cryst.	(13)	272.9	Modified	0.205	16	74	167.4
15	Debromination fourteen times cryst.	(12)	272.8	Modified	0.206	16	67	166.4
16	Debromination six times cryst.	(13)	272.9	Modified H_3PO_4	0.222	16	67	177.9
17	Debromination six times cryst.	(13)	272.9	Modified Pyr. SO_4	0.197	16	64	181.6

* Numbers in this column refer to Table I of our previous report.

Similar values on various specimens of linolenic acid, crystallized from 6-14 times, and employing five different preparations of thiocyanogen reagent gave the following values: 165.5, 165.6, 165.9, 166.3, 166.4 (No. 15, Table V), 166.5 (Table IV), 166.7 and 167.2 (average of Nos. 12, 13, 14, Table V). The average of these values is 166.3, which again is somewhat lower than by the official method and higher than by the Kaufmann method.

The average value for linoleic acid, previously noted, 96.6, is exactly that for linoleic acid No. 5 as determined by the modified procedure. The average value for linolenic acid 166.3 compares favorably with 166.4, as determined on specimen No. 15.

In the preparation of No. 5 and No. 15 by repeated crystallization, Filtrate II, in each case, (17) was examined; they were found to give thiocyanogen numbers of 95.2 and 163.2 respectively. These values would appear to show that the isomers present in nearly equal amounts (as shown by calculation from the tetra- and hexabromide values) do not react at appreciably different rates.

Analysis of Synthetic Mixtures of Fatty Acids

From the thiocyanogen values 89.9, 96.6 and 166.3 for oleic, linoleic and linolenic acids and the theoretical iodine numbers for these acids, corrected simultaneous equations can be set up for analyzing mixtures of fatty acids. In the equations which follow,

S_0 = % saturated acids
S_1 = % oleic acid
S_2 = % linoleic acid
S_3 = % linolenic acid
TV = thiocyanogen value
IV = iodine number (Wijs, 1 hr.)

In mixtures containing only linoleic, oleic, and saturated acids,

$$S_1 = 2.3821\ TV - 1.2701\ IV$$
$$S_2 = 1.1825\ (IV - TV)$$
$$S_0 = 100 - (S_1 + S_2)$$

In mixtures containing all three unsaturated acids and saturated acids, S_0 being known,

$$S_3 = 1.589\ TV - 0.116\ IV - 1.324\ (100 - S_0)$$
$$S_2 = 1.329\ IV - 3.199\ TV + 1.681\ (100 - S_0)$$
$$S_1 = 100 - (S_3 + S_2 + S_0).$$

Five synthetic mixtures of fatty acids of known composition were made up and, after determination of iodine and thiocyanogen numbers, their composition was calculated by the use of modified equations. In mixtures I, II and IV, the acids employed were specimens described in Table II. Since the oleic acid contained appreciable amounts of saturated acids and linoleic acid, the known compositions of these mixtures have been corrected. The palmitic acid used was a specimen of iodine value less than 0.2. The oleic acid in mixture V was about 99.8 percent pure; iodine number 89.9; TV, 89.7. Linoleic and linolenic acids in mixtures III and V were the highly purified specimens which were used in establishing the standard thiocyanogen values. Data and results are given in Table VI.

TABLE VI

The Analysis of Known Mixtures of Fatty Acids from Thiocyanogen-Iodine Number Data

	Composition of Mixture			
	Palmitic S_0	Oleic S_1	Linoleic S_2	Linolenic S_3
MIXTURE I Iodine No. 90.0 $(SCN)_2$ No. 79.4				
Acids weighed out, g.	0.300	2.396	0.300	....
Known % of pure acid	12.6	75.0	12.4	....
% calc. by Kaufmann equation	11.8	76.4	11.8	....
% calc. by corrected equation	12.6	74.7	12.7	....
MIXTURE II Iodine No. 130.9 $(SCN)_2$ No. 79.1				
Acids weighed out, g.	0.453	0.751	1.805	....
Known % of pure acid	15.9	23.4	60.7	....
% calc. by Kaufmann equation	12.7	30.0	57.3	....
% calc. by corrected equation	16.5	22.2	61.3	....
MIXTURE III Iodine No. 99.3 $(SCN)_2$ No. 65.9				
Acids weighed out, g.	1.003	1.005	1.349	....
Known % of pure acid	29.9	29.9	40.2	....
% calc. by Kaufmann equation	27.0	36.1	36.9	....
% calc. by corrected equation	29.7	30.9	39.4	....
MIXTURE IV Iodine No. 235.6 $(SCN)_2$ No. 135.9				
Acids weighed out, g.	....	0.0	1.197	1.811
Known % of pure acid	....	0.0	39.8	60.2
% calc. by Kaufmann equation	....	—9.8	60.6	49.2
% calc. by corrected equation†	....	—1.7	46.5	55.2
MIXTURE V Iodine No. 191.1 $(SCN)_2$ No. 122.8				
Acids weighed out, g.	....	1.003	1.003	1.407
Known % of pure acid	....	29.4	29.4	41.2
% calc. by Kaufmann equation	....	24.8	40.0	35.2
% calc. by corrected equation	....	30.4	29.1	40.5

† The linolenic acid employed in this mixture had a thiocyanogen number of 162.7. It seems probable that the presence of this acid is the source of error in the calculated values, because these are much closer to the theoretical when equations derived from this thiocyanogen number are used in solution of the mixture.

The results in Table VI show, in agreement with several reports, cited previously, (6-13) that the original Kaufman equations are based on fundamentally erroneous thiocyanogen values. When, however, the equations are corrected in accordance with actual observed thiocyanogen values, the composition of known mixtures can be determined with satisfactory accuracy.

Our accepted thiocyanogen values agree best with those of Kass *et al.*, being only very slightly lower for both acids, a difference which may in part be ascribed to the use of a slightly lower temperature. On the other hand, our values are somewhat higher than those proposed by Hilditch and Murti, These investigators noted "low" thiocyanogen values for acids which had been stored for some time, a phenomenon which we have also observed, more especially with some of our earlier specimens of acids. In specimens carefully preserved in evacuated sealed ampules at —20°, we have usually had no difficulty in this respect. It is difficult to see how an acid may change during storage without fall in iodine number and still show a drop in the thiocyanogen value.

We may conclude from our results and from those reported from other laboratories that the thiocyanogen method is a valuable aid in fat and oil analysis but it is clear that the method should be studied further with the objective of setting up standardized details of procedure which can be agreed to by the investigators interested in this technic. Reference samples of acids should be prepared preferably several specimens of each, and results of analysis from different laboratories compared. The official Committees on fats and oil analysis of the American Chemical Society and the American Oil Chemists Society might well undertake such a cooperative problem.

Summary

The thiocyanogen reagent may be stabilized by storage at temperatures of 3° or less. The reaction of thiocyanogen with highly purified linoleic and linolenic acids has been studied under many variations of experimental conditions. Reaction rates of thiocyanogen with oleic, linoleic and linolenic acids at 3°, 16° and 25° and with variable excess of reagent are described. Oleic acid gives a maximum thiocyanogen absorption which is apparently equal to the iodine number, within 3-6 hours. The reaction with linoleic acid is rapid up to 3 hours and shows a slow constant increase thereafter. A modified procedure of analysis, similar to the official method, employs a 0.2 N thiocyanogen reagent, containing 10 percent carbon tetrachloride and a reaction temperature of 16°. Results of analysis of several specimens of acids by several preparations of reagent shows an average thiocyanogen value of 96.6 for linoleic acid and 166.3 for linolenic. Simultaneous equations, derived from these values, were used in the analysis of five mixtures of known composition with excellent results.

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Report of the Olive Oil Committee 1940-41

THIS committee has had under consideration two matters: first, the application of the approved Fitelson Test for the detection of teaseed oil in olive oil to those olive oils giving interfering colors and secondly, the value of the U.S.P. cold test on edible olive oils.

We found, in our work on the Fitelson Test, that the color of some olive oils, especially the inedible or so-called commercial olive oils, as a class, tended to mask the characteristic reddish coloration developed by ether in the final stage of this test. To overcome this difficulty, the following modification, suggested by the chairman, has been tried, found satisfactory, and is recommended for adoption as an alternative when conditions are met with which show the necessity for clearer colors.

"Saponify 5 grams of the oil to be tested plus 5 grams of colorless mineral oil or "albolene" with excess alcoholic potash (or 5 cc. of 50% KOH in about 30 cc. of alcohol). Brisk boiling for from 10 to 15 minutes usually suffices. Pour the liquid into a separatory funnel, add an equal volume of water, shake and allow the layers to separate. Draw off and reject the lower soap solution. Wash the oily layer with water several times to get rid of the soap. Finally dry the oil over anhydrous sodium sulphate and filter for the test. Seven drops of this oil are used in the regular way for the Fitelson test, using, however, standards for comparison made up with mineral oil in the same way, which can be kept permanently."

The second matter under investigation was a result of numerous requests from members of the olive oil trade because several rejections of olive oil occurred in the past few years by reason of the cold test in spite of the fact that all other requirements were met.

The U. S. P. XI on page 263, under tests for identity and purity (!) of the olive oil, states that "when cooled to from 10° to 8° C., olive oil becomes *somewhat* cloudy and at about 0° C. it *usually* becomes a whitish granular mass." (The italics are mine.)

This test has been in the Pharmacopeia since 1880 without change. Whether conditions of manufacture have actually altered the situation which may once have existed, or, perhaps because the test in spite of its loose wording is being applied seriously, the fact remains that some undoubtedly pure olive oils have been turned down on this test alone.

The test give no time limit so that a reasonable length of time must be read into it. It is also placed in the category of a purity or identity test, which is rather difficult to understand as "cold" tests are usually considered indices of quality or grade. This is the case with winterpressed cottonseed oil, for example.

However, the committee took the matter up seriously and some of the members have tried the cold test on quite a number of oils. The consensus of opinion is that the test is worthless as a test for identity and purity of olive oil and that from any standpoint it is of no particular value since the olive oils themselves vary so greatly in composition.

OLIVE OIL COMMITTEE:

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