A New Accelerated Holding Test Involving Aeration of Oils in Iron Tubes

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THE active oxygen method (A.O.M. test) described by King, Roschen, and Irwin (1) and modifications thereof by Riemenschneider, Turer, and Speck (2) as a means of evaluating the keeping qualities of oils and fats have been used by the vegetable oil and animal fat industries for many years. This method depends upon the aeration of oils and fats (usually freshly processed) in glass at 98°C. with the determination of the progressive increase in peroxide value as an index of oxidative deterioration. The time in hours required for the attainment of a certain peroxide value associated with an organoleptically detected rancidity, *viz.*, a peroxide value of 100 milliequivalents per kilogram of hydrogenated vegetable oil, is the value reported. In citing such data, the possibility of subsequent metal pickup changing the value is neglected. Oils and fats are transported from refiner to bulk users in sheet iron tank cars. Users cream the fat with other ingredients in sheet iron mixers or fry with these products in sheet iron vats. Then why conduct A.O.M. tests only in glass apparatus? Certainly, such tests on fats relatively free of metals can fail to predict which fat is better protected against subsequent metal pickup.

The object of the present report is to present an accelerated holding test for evaluating the resistance of oils and fats to oxidative deterioration catalyzed by prooxidant metals. By simply replacing the glass tubes in the conventional A.O.M. test with iron tubes of similar design, the factors of iron contamination and contact metal catalysis are now brought into play. The precision of the modified A.O.M. test is good when the iron tubes are properly prepared. The results on an absolute and relative basis obtained in testing fats by the conventional and modified A.O.M. stability tests show striking discrepancies.

Experimental

Test methods. The assembly for the A.O.M. test used in the current studies was that described by King, Roschen, and Irwin (1) except that the glass tubes in which the fats were aerated were now replaced by iron tubes made by welding a small iron plate to the bottom of a 7¾-in. length of ¾-in. iron pipe. An aqueous solution of potassium dichromate and sulfuric acid, each in 2% concentration (3) was employed for washing the incoming air in place of the potassium permanganate solution (1).

The preparation of the iron tubes prior to evaluation of the test fats has proved to be of primary importance, and the procedure to be described must be carefully followed in order to obtain reproducible results. The tubes are cleaned by placing them in a 1 + 1 solution of concentrated hydrochloric acid and water for three hours. The tubes are then scrubbed with a hard bristle brush in order to remove loose iron, rinsed well with water, followed by acetone and finally ether, and blown dry with nitrogen. The tubes are next rinsed twice with the liquid test fat. Twenty ml. of test fat are added to each tube for the test. In the event the test cannot be run immediately after the tubes have been prepared, the tubes are filled with the test oil and held at the melting point of the oil until ready for use. This oil is discarded, and the tube is rinsed twice with fresh portions of test oil and wiped with cheesecloth; and 20 ml. of the fresh oil are added to the tube for the accelerated test.

Approximately 2-gm. samples of the oils aerated at 98°C. were taken at periodic intervals for determination of the peroxide value and for flavor scoring. The peroxide values were determined on a 0.2- to 0.3-gm. sample by the method of Riemenschneider, Turer, and Speck (2) and calculated as milliequivalents per kilogram of fat. Acceptability of oil flavor as employed in this study means that the oils were still edible; the cooked flavor which develops during aeration of the oils at the elevated temperature in both glass and iron tubes was discounted.

For comparative purposes A.O.M. tests of the same fats, using the conventional glass tubes, were employed. In some cases iron as iron stearate was dissolved in the oils aerated in the glass tubes.

TABLE I

Precision of A.O.M. Test Conducted on Commercial Shortenings Aerated at 98°C. in Glass and Iron Tubes

Code	Identity[a]	Glass Tubes			Iron Tubes		
		(a)	(b)	Average	(a)	(b)	Average
		Hours to a 100 m.e./kg. peroxide value					
A	Lard plus 0.02% BHA, 0.01% PG and 0.005% CA	35	35	35	9	14	12
B	Lard plus 0.02% BHA and 0.005% CA	54	55	55	18	18	18
C	Hydrogenated vegetable oil	81	83	82	35	30	33
D	Hydrogenated vegetable oil	98	98	98	28	30	29
E	Hydrogenated vegetable oil	106	112	109	38	37	38
F	Hydrogenated vegetable oil	118	117	118	18	23	21
G	Hydrogenated vegetable oil	120	121	121	58	61	60
H	Hydrogenated vegetable oil	137	141	139	59	64	62
I	Hydrogenated vegetable oil	142	138	140	23	28	26
J	Hydrogenated vegetable oil	148	154	151	21	25	23
K	Hydrogenated vegetable oil	201	200	201	52	52	52
L	Hydrogenated vegetable oil plus 0.08% IC	208	216	212	174	178	176
		Reproducibility of the Values Obtained in Hours					
Based upon a single analysis		±2.7(1 S.D.)			±2.7(1 S.D.)		
Based upon the average of duplicate analyses		±1.9(1 S.D.)			±1.9(1 S.D.)		

[a] BHA = butylated hydroxyanisole; PG = propyl gallate; CA = citric acid; IC = isopropyl citrate esters, predominantly monoisopropyl citrate.

TABLE II

Precision of the A.O.M. Test Conducted on a Hydrogenated Vegetable Shortening Containing Various Antioxidants and Aerated at 98°C. in Glass and Iron Tubes

Antioxidant	Glass Tubes			Iron Tubes		
	(a)	(b)	Average	(a)	(b)	Average
	Hours to a 100 m.e./kg. peroxide value					
None	68	73	71	39	40	40
0.02% butylated hydroxyanisole	136	136	136	44	41	43
0.005% citric acid	136	136	136	61	56	59
0.006% propyl gallate	112	116	114	44	47	46
0.10% lecithin	133	138	136	33	33	33
0.08% isopropyl citrate	150	150	150	154	156	155
0.005% citric acid + 0.006% propyl gallate	290	294	292	51	56	54
0.10% lecithin + 0.006% propyl gallate	237	243	240	47	53	50
0.02% butylated hydroxyanisole + 0.006% propyl gallate + 0.005% citric acid	290	289	290	120	115	118
0.02% butylated hydroxyanisole + 0.006% propyl gallate + 0.08% isopropyl citrate	320	325	323	278	285	282
	Reproducibility of the Values Obtained in Hours					
Based upon a single analysis	±2.7 (1 S.D.)			±3.0 (1 S.D.)		
Based upon the average of duplicate analyses	±1.9 (1 S.D.)			±2.1 (1 S.D.)		

Iron analyses were conducted on a number of oil samples by the A.O.A.C. orthophenanthroline method (4), following exhaustive hot acid extraction of a 20-gm. oil sample and using the concentrated acid extract as the test solution. The standard deviation, based upon recovery experiments, has shown that this method is accurate to within ±0.1 p.p.m. of iron.

Reproducibility of the conventional and modified A.O.M. tests. In Table I are listed values obtained in duplicate when commercial shortenings were aerated at 98°C. in glass or iron tubes. It will be noted that the reproducibility of values, expressed in hours, based upon a single analysis or one run in duplicate was the same in the tests conducted on the oils in glass or iron tubes. Actually the precision of the results on the oils in the iron tubes was poorer in relation to the number of hours for completion of the test; in iron tubes the oils exhibited striking decreases in A.O.M. values. The only exception was Shortening L, the product containing 0.08% mixed isopropyl citrate esters predominantly monoisopropyl citrate (5). The lard samples (A and B) were poorly protected in the iron tubes despite fortification with antioxidants. The variability in results between different shortenings is attributed to variability in concentration of added but not declared metal sequestering agents; compare, for example, results obtained on Shortening G with those on Shortening J. It is very likely that Shortening G had been treated with citric acid at some stage in processing while Shortening J had not had the benefits of such treatment. But even in the case of Shortening G (or H for that matter), protection against the prooxidant effects of iron was not impressive. The striking results obtained with Shortening L support the conclusion that the limited solubility of citric acid in oils restricts its effectiveness under the method of test. Ease of use and ready solubility very definitely favor the esters of citric acid (5).

In order to check some assumptions made above on the likely presence or absence of metal sequestering agents in the commercial shortenings and in order to check further into the reproducibility of the modified A.O.M. test, a study was conducted on one shortening before and after supplementation with the more popular antioxidants alone and in combinations in the customary concentrations used commercially. The results of this study are summarized in Table II.

The reproducibility of values obtained by the conventional and the modified A.O.M. test in this series was comparable to that obtained with the different shortenings (see Table I). Statistical evaluation of the combined data in Tables I and II show that the values (averages of duplicates) by either the conventional or modified methods are reproducible to within ±2 hours, actually ±1.9 and ±2.0 hours, respectively, for one standard deviation. The standard deviation of a single value is 2.7 hours in the former and 2.8 hours in the latter test.

It will be noted from the results obtained with the hydrogenated vegetable oil in glass tubes that various antioxidant mixtures were apparently superior to the isopropyl citrate alone. This however was not the case when the tests were conducted in iron tubes. Under these circumstances the high concentration of isopropyl citrate conferred a degree of stability strikingly superior to that attained with the other antioxidants, alone and in combinations. The best antioxidant combination appeared to be butylated hydroxyanisole, propyl gallate, and isopropyl citrate. The latter replaced the small concentration of citric acid customarily used in antioxidant combinations.

Flavor scorings conducted periodically on the fats described in Tables I and II during progressive oxidative deterioration were in very good agreement with the corresponding peroxide values. It was established that the peroxide values accepted as end-points in the conventional A.O.M. test, *i.e.*, 100 milliequivalents per kilogram for hydrogenated vegetable oils and 40 for hydrogenated lard, were equally applicable to the oils aerated in the iron tubes. Since the curve of increasing peroxide value (or rancidity) was so steep for the lard products subsequent to the attainment of a 40 peroxide value, the reporting of all values obtained to the common end-point of 100 was preferred.

Significance of the modified A.O.M. test. It was anticipated in initiating the present study that the aeration of oils in iron tubes would provide a dynamic test system for evaluating the resistance of the oils to oxidation catalyzed by a prooxidant metal. Progressively increasing contamination of the aerated oil with iron in the modified A.O.M. test was expected. In practical operations an oil or shortening does not become contaminated with a specific quantity of iron or other prooxidant metal but exhibits a progressive pickup of such metals as the oil is progressively exposed to metal equipment in transportation, storage, and use. The addition of a specific quantity of iron, as iron stearate, to the oil in the conventional glass tube cannot simulate adequately what occurs under conditions of use.

In order to check the above assumption, a study was conducted to determine the rate of iron pickup in oils aerated in iron tubes and to compare peroxide development in such oils with that noted for the same oils supplemented at one time with known quantities of iron and then aerated in glass tubes. The influence of the mixed isopropyl citrate esters, predominantly monoisopropyl citrate (5), in stabilizing the aerated oils in both test systems was also evaluated.

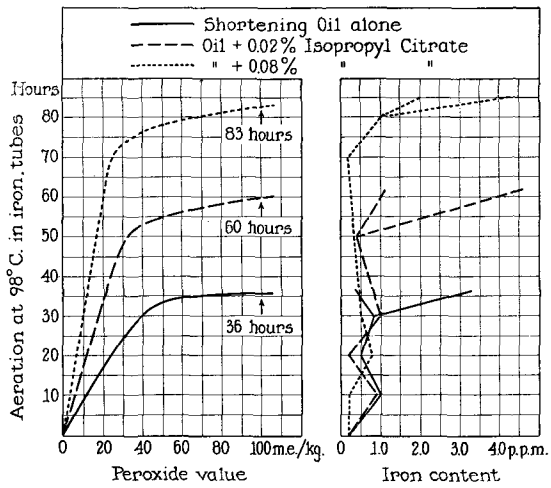

FIG. 1. Peroxide development and iron pickup during aeration in iron tubes of a hydrogenated vegetable oil with and without added isopropyl citrate esters, predominantly monoisopropyl citrate.

In Figure 1 are presented the results obtained in conducting A.O.M. tests on a hydrogenated vegetable oil aerated in iron tubes. This particular oil had an A.O.M. value of 79 hours according to the conventional test and 125 hours when protected with isopropyl citrate in either 0.02 or 0.08% concentration. The marked increase in the susceptibility of the oil to oxidation when aerated in the iron tube was again noted; 36 hours in contrast to the 79 hours obtained when the all-glass apparatus was employed. Isopropyl citrate protected the oil to such a degree that, at the 0.08% level, a value for the oil in the iron tube was obtained at least as good as that for the same oil without isopropyl citrate in the glass tube. This concentration of metal sequestering agent cannot be obtained with free citric acid.

Also plotted in Figure 1 are the iron values of the oils that had been aerated in the iron tubes. Because of the limited sensitivity of the colorimetric test for iron, each oil sample analyzed came from a different tube. This explains the erratic iron figures of from 0.2 p.p.m. to 1.0 p.p.m. as the oils were progressively aerated to the point of accelerated peroxide development. The variations in iron values were not due to imprecision of the test method. The method is accurate to within ± 0.1 p.p.m. of iron, *i.e.*, the standard deviation based upon recovery experiments. It was repeatedly observed during the periods of moderate increase in peroxide value, covering almost 90% of the aeration periods, that fairly good agreement between peroxide values of oils from duplicate tubes was obtained despite wide variations in the iron content of these oils. At the break point in the test, when the peroxide values sky-rocketed, there were marked but still variable increases in iron content of the oils; the variability in the iron values is indicated in Figure 1 by the branching of the curves showing iron pickup. Indeed, when the oils were aerated for another hour or so, yielding peroxide values of 500 to 600, iron values as high as 30 p.p.m. were obtained. But here also there was poor correlation between peroxide value and iron content. The free fatty acid values paralleled to some degree the iron figures. During the major portion of the aeration period the free fatty acid values were small and variable from 0.06 to 0.20%, but at the point of sky-rocketing peroxide values the free fatty acid values were increased to about 1.3%. In the oils in the glass tubes the free fatty acid values increased by only 0.1%. The increase in free fatty acid values of the oils in the iron tubes at the terminal stages of the test may very well be responsible for the increased iron contamination.

In contrast to the results noted with the oils aerated in iron tubes are the findings in Figure 2 and

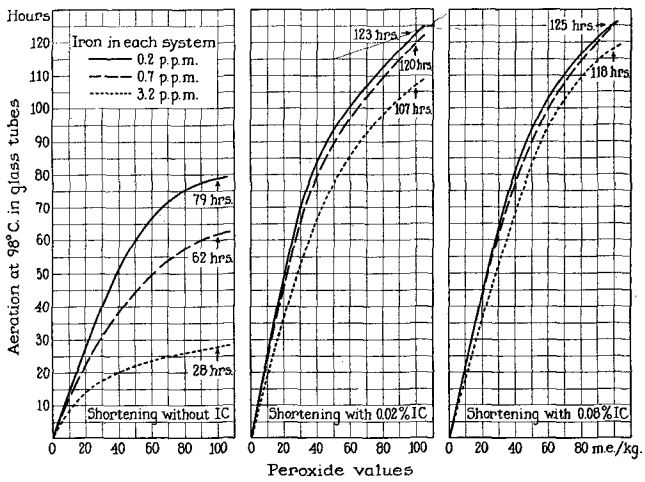

FIG. 2. Peroxide development during aeration in glass tubes of a hydrogenated vegetable oil with and without added isopropyl citrate (IC, predominantly monoisopropyl citrate) and before and after supplementation at one time with known quantities of iron as iron stearate.

obtained with the same oils aerated in glass tubes before and after supplementation at one time with known quantities of iron, as iron stearate. It will be noted that the acceleration in peroxide development at the terminal stages of the test was not as intense in glass tubes. The results on the oil without isopropyl citrate aerated in the iron tube (see Figure 1) most closely approximated the findings plotted in Figure 2 for the oil with 3.2 p.p.m. of iron, an iron value far in excess of that obtained during the major period of aeration of the unprotected oil in the iron tube. Isopropyl citrate at the two levels employed performed far more efficiently in protecting the oils against oxidation in glass tubes despite the far higher iron contents. Flavor scorings of the aerated oils in both glass and iron tubes confirmed the A.O.M. values presented.

It will be noted that peroxide values have been plotted on the abscissa and the hours aerated on the ordinate in both Figures 1 and 2. In so plotting the data, an index of overall keeping quality could be obtained. To evaluate the keeping quality by determining the number of hours to attain a specific

peroxide number (100 m.e./kg.) does not take into account the rate of peroxide development during the induction period. For a more critical evaluation the area under the curves presented in Figures 1 and 2 were measured. These values were expressed relative to the area obtained with the system "free" of iron set at 100; the basic oil in glass was rendered "free" of iron by the 0.08% isopropyl citrate. By this device, overall keeping quality of the oils was calculated.

TABLE III

Significance of A.O.M. Test Values on Shortenings With and Without Added Iron

Test system	Shortening with isopropyl citrate					
	0.0%	0.02%	0.08%	0.0%	0.02%	0.08%
Oil in glass tubes	Hours[a]			Overall quality[b]		
0.0 p.p.m. Fe[c]	125	125	125	100	100	100
0.2 p.p.m. Fe[d]	79	123	125	64	100	100
0.7 p.p.m. Fe[d]	62	120	125	48	95	98
3.2 p.p.m. Fe[d]	28	107	118	24	84	90
Oil in iron tubes	Hours[a]			Overall quality[b]		
Variable Fe content[e] of 0.2-1.0 p.p.m.	36	60	83	32	58	85
Fe content of oil in glass to give same A.O.M. picture for oil in iron tubes	p.p.m. of iron					
	2.5	Well above 3.2	Well above 3.2	2.0	Well above 3.2	More than 3.2

[a] To a peroxide value of 100 m.e./kg. of fat.
[b] Area under curve, plotting peroxide value versus hours of aeration at 98°C.; the area obtained with the system "free" of iron was set at 100 to obtain relative values for overall keeping quality.
[c] The basic oil in glass rendered "free" of iron by the 0.08% isopropyl citrate.
[d] Quantity of iron in the refined deodorized oil; the other values were obtained after supplementation with iron stearate.
[e] Just prior to the termination of the test.

The values for overall keeping quality have been listed in Table III. The hours required to attain the end-point in the A.O.M. tests are also presented for these test oils containing a variable iron content. It is apparent from the evaluation of results in Table III that the greater susceptibility of oils to oxidative deterioration in iron tubes is not due to the prooxidant effects of dissolved iron but primarily to contact metal catalysis and that isopropyl citrates minimize the deleterious effects due to both dissolved iron and contact metal catalysis. It would follow therefore that the present modification of the A.O.M. test is superior to the conventional A.O.M. test (even when the latter involves the addition to the oil of a specific amount of iron as a fatty acid salt) in evaluating the potential value of metal sequestering agents.

In Table IV are presented results obtained when another hydrogenated vegetable oil was aerated in iron tubes to a point just prior to sky-rocketing peroxide values. The oils were then transferred to glass tubes, and the A.O.M. test was carried to completion. From the results obtained it has been concluded that a marked instability has been imparted to the oils even during the early period of aeration in iron tubes and that isopropyl citrate is strikingly effective in protecting the oils during this period. It is postulated that the tocopherols (very weak acids) in the oils are either adsorbed by the metal wall and rendered ineffective or destroyed during the period of aeration. Thus the oil used in the A.O.M. test No. 6 may be regarded after the aeration in the iron tubes to be an essentially tocopherol-free oil. In those oils containing the isopropyl citrate esters, predominantly

TABLE IV

Instability Imparted to Shortening During Early Period of Aeration at 98°C. in Iron Tubes and Protective Influence of Isopropyl Citrate Esters (IC) in Minimizing This Effect

A.O.M. test	IC added	Aeration in tubes	Peroxide value attained	Hours Required	
				In each tube	Total
	per cent		*m.e./kg.*		
1	0.00	Iron	100	19	19
2	0.00	Glass	100	74	74
3	0.08	Iron	100	92	92
4	0.08	Glass	100	150	150
5	0.00	Iron	10	4	22
		and then glass	100	18	
6	0.00	Iron	39	17	
		and then glass	100	3	20
7	0.08	Iron	20	66	
		and then glass	100	63	129
8	0.08	Iron	30	72	
		and then glass	100	40	112

monoisopropyl citrate (a very much stronger acid), the competition for metal between tocopherols and monoisopropyl citrate is in favor of the latter. Under such circumstances the tocopherols should be better protected and therefore remain in the oil as effective antioxidants for longer periods of time. Studies dealing with the possible mechanisms involved in tocopherol retention are currently in progress.

It is recognized that the ratio of metal surface to fat in the iron tube test described in this paper is enormously greater than would be encountered in actual practice. However it should be remembered that the new test is an accelerated holding test designed to rate the effectiveness of antioxidants, particularly metal sequestering agents, in protecting oils. For an accelerated holding test the high ratio of metal to oil is not only desirable but required, just as high-temperature aeration of the oils in the conventional A.O.M. test is desirable and required for prompt evaluation of oils. The iron tube test undoubtedly overemphasizes the prooxidant effect of metals in promoting deterioration of oils in commercial operations; on the other hand, the conventional A.O.M. test fails to take into account the effects of dissolved metals and metal surfaces on oils under conditions of use. The relative keeping times of fats and oils in actual practice probably fall somewhere between these two extremes. Thus both tests have virtue in evaluating oils; the uniformity of values by the conventional A.O.M. test conducted on different batches of a given oil without antioxidants and metal sequestering agents reflects the reproducibility of the oil produced in the plant with respect to inherent resistance to oxidative deterioration; the values by the iron tube test reflect the degree of resistance of the oil to oxidation following extreme exposure to metal contamination and contact metal catalysis. Certainly, the iron tube test will predict which oil is better protected against the prooxidant effects of metals subsequently encountered.

Summary

A modification of the conventional A.O.M. test for evaluating the keeping qualities of fats and oils has been presented. This involves the substitution of an iron tube for the glass tube normally used in this test. The object in this change of procedure is to

permit evaluation of oils under conditions promoting oxidation catalyzed by metal pick-up and by contact with metal surfaces, situations encountered in commercial operations. It has been shown that a substantial lowering of the A.O.M. values is obtained when the modified test is used.

Data are presented on the reproducibility of the method for evaluating the stability of shortenings and the protective influence of antioxidants. Isopropyl citrate esters, predominantly monoisopropyl citrate, have proved to be superior to other metal sequestering agents in the test described since the esters can be readily added to oils in sufficiently high concentrations to be effective. Isopropyl citrate esters protect the oils not only against the prooxidant effects of dissolved iron but also against contact metal catalysis.

The relative keeping times of fats and oils in actual practice probably fall somewhere between the two extremes predicted from the results of the conventional and the modified A.O.M. tests. Both tests have virtue in evaluating oils; the reproducibility of values by the conventional test, conducted on different batches of a given oil without antioxidants and metal sequestering agents, reflects the reproducibility of the oil produced in the plant with respect to inherent resistance to oxidative deterioration; the values by the iron tube test reflect the degree of resistance of the oil to oxidation, following extreme exposure to metal contamination and contact metal catalysis.

All of the oils tested in the present study have been subjected to flavor evaluations in addition to serial determinations of peroxide value. The former have supported the latter relative to establishing the end-point or first sign when the oils develop rancidity.

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The Composition of Coffee Oil and Its Component Fatty Acids

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COFFEE is a favorite drink almost all over the world. The water-soluble portions of the coffee bean are thus utilized, and the rest is thrown out as waste. The coffee grounds are mainly composed of proteins, carbohydrates, and lipids. About 20-25% of the dry weight of the water-extracted grounds is recoverable as oil. Coffee grounds in enormous amounts are a product of the soluble coffee industry and actually present a problem for industrial disposal. However they are a potential source of protein and carbohydrate and also of oil of possible edible and other uses. The object of the present work was to investigate the composition of coffee oil in relation to its possible uses. The magnitude of the soluble coffee industry makes recovery of the oil practical, provided there are industrial outlets for its use.

Previous work on coffee oil is meager and conflicting. Robiquet and Boutron (1) in 1837 showed that the coffee-bean contains about 10% of fat, which is extractable with ether. From that time until the end of the past century various reports dealing with coffee fat were published by Rochleder (2), Chech (3), De Negri and Fabris (4), Hilger and Tretzel (5), Hilger (6), Spath (7), Herfeldt and Stutzer (8), and Warmier (9). In most cases these investigators reported the determination of certain constants and partial analyses of the fat; few systematic attempts were made to isolate or identify the individual fatty acids. Juckenack and Hilger (10) however made an extensive study of the fat obtained from various kinds of coffee, both raw and roasted, and reported that the fat consisted largely of glycerides of oleic acid and only minor quantities of glycerides of palmitic and stearic acids. Meyer and Eckert (11) fractionally crystallized the acids from the oil of decaffeinized coffee and reported about 40% of saturated acids, consisting of capric (0.5%), palmitic (25-28%), daturic (1-1.5%), and carnaubic (10%) acids; the unsaturated acids were stated to be composed of 2% oleic acid and 50% linoleic acid. Von Noel (12) investigated six oils from several kinds of coffee. The limits of constants observed were: d, 0.9288-0.9453; saponification number, 176.1-179.15; iodine number, 87.1-92.4; Reichert-Meissl number, 0.52-0.66; Polenske number, 0.2-0.25; unsaponifiables, 6.53-13.49%. The fatty acids of the oil were found to be palmitic, daturic carnaubic, oleic, and linoleic acids. He stated that the fatty acids suffer little change during roasting.

The most important work on coffee oil is that of Bengis and Anderson (13), who studied the component fatty acids of coffee-bean oils of different types (green bean, freshly roasted, stale roasted). Separation of the crude acids by the lead soap method gave 54.4% of liquid acids, consisting of linoleic acid 29.5%, oleic acid 21%, and petroleum-ether-insoluble acid designated as hydroxy unsaturated acids (C_{18} or otherwise) 4%; and 37.5% of solid acids, including palmitic 29.2%, stearic 6.4%, and tetracosanoic 1.8%. They were able to identify tectracosanoic acid but found no C_{20} or C_{22} acids. More recently, Bauer and

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