137
.129
THS

ISOLATION AND PROPERTIES OF ANTIOXIDANT FRACTIONS FROM SEED OILS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Robert W. Kindle

1942

ISOLATION AND PROPERTIES OF ANTIOXIDANT FRACTIONS FROM SEED OILS

by

Robert W. Kindle

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

1942

T 57-7
K 51

The writer wishes to sincerely thank Professor C. D. Ball and Mr. Carroll King for their kind assistance in this work.

Robert W. Kindla

143862

TABLE OF CONTENTS

Introduction

Antioxidants are those substances which inhibit the oxidation of an oxidizable material, so that the length of time required for oxidation is increased. For the naturally occurring antioxidants isolated from vegetable oils the term "inhibitol" has been suggested, the ending -ol inferring hydroxyl groups. Such groups have been found present in these natural compounds.

It was the purpose of this study to isolate these natural occurring antioxidants from several vegetable oils and attempt to concentrate an active portion by fractionation of the isolated material. The work is incomplete so far as isolating a chemical entity is concerned, but a definite concentrating of the active material was accomplished.

The term fat or oil is referred to in this work as being the material which can be extracted from plants or plant products or from animal matter with ethyl ether, petroleum ether, or other similar solvents, and to consist essentially of mixtures of glycerides. Both terms, oil and fat, are frequently used interchangeably in scientific literature.

Historical

Although the oils in ripening seeds and nuts are exposed to extreme oxidative conditions of air, light, heat, and moisture, they seldom become rancid. This fact points to some mechanism which protects the natural oils from oxidation, either, by keeping at a low level, the oxygen in contact with the oil or by forming a substance which tends to inhibit oxidation.

Although Moureu and Dufraisse (1) in 1922 emphasized the importance of antioxygens, the isolation of substances inhibiting oxidation from fats and oils was not discovered until several years later. Anderegg and Nelson (2) in 1926 first noticed that a small amount of wheat embryo oil, when added to the animal diets they were using, protected the cod liver oil in the diet from rapid oxidation. Mattill (3) in 1927, also working with animal diets, noted the action of wheat germ oil in protecting vitamins A and E from oxidation. He and his associates (4,5) in further studies of fat oxidation in relation to the vitamins, found that when fats were combined in diets, they tended to mutually influence the auto-oxidizability. Thus, as an example, the induction period of a mixture of lard (27 hours) and cod liver oil (3 hours) was approximately 9 hours.

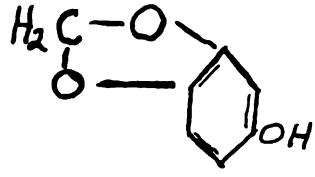
Mattill and Crawford (6), working with corn oil, noted that each step of the commercial purification of the oil decreased the induction period. They found that

the antioxidant fraction could be concentrated in the unsaponifiable fraction of the oil and attributed some of the action to the sterols present. Later, this observation regarding the activity of the sterols was refuted (7).

Olcovich and Mattill (8,9,10) in 1931 described a crystalline antioxidant isolated by fractionation with organic solvents from the unsaponifiable material of the lipids of lettuce. They could not concentrate the antioxidant by crystallization but finally obtained, by fractional distillation in vacuo, a white solid. The oily fraction distilling from 160°- 180°C. at 0.001 mm., on cooling yielded crystalline material which, when purified, proved to have marked antioxidant capacity. This material formed colorless crystals from acetone, leaflets from chloroform, m.p. 142°C., was very soluble in alcohol, ether, acetone, and dilute alkali, moderately soluble in chloroform and benzene, and insoluble in petroleum ether and water. They assigned the formula $C_{13}H_{14}O_5$ and noted that the compound contained hydroxyl groups which were responsible for the activity, since its inhibiting ability was lost on acetylation but regained on hydrolysis of the acetate. In this separation they definitely segregated vitamin E and the antioxidant material into two different fractions - neither possessing the characteristic property of the other. Their active antioxidant fraction was concentrated in methyl alcohol when the substance was partitioned between petroleum ether and 92% methyl alcohol.

Gossypol, a crystalline compound isolated from cottonseed oil, has been shown to have marked antioxidant activity (7,11). This compound contains several phenolic groups which are sufficient to account for its activity. Its structure was postulated as a binaphthyl derivative, however, the positions of the methyl groups in the molecule have not been definitely clarified (12).

Sesamol, the phenolic substance responsible for the Villavechia and Baudoin color tests of sesame oil, was found to be an effective inhibitor in fats and oils (13). It was thought that perhaps the gradual liberation of sesamol from sesamolin, a glucoside of sesamol, created a favorable environment for preventing oxidation. Sesamol possesses the formula,



and has been synthesized by Boeseken, Coden, and Kip (14).

By resynthesizing glycerides from distilled fatty acids obtained from vegetable oils, Hilditch and Sleightholme (15) showed induction periods of greater length in the natural oil than in the synthesized glycerides. The evidence obtained was conclusively in favor of the view that the induction period phenomenon was caused by the antioxygenic action of the non-glyceride components of the oils.

In 1934 Bradway and Mattill (16) fractionated the unsaponifiable material from tomato, carrot, and wheat germ oils, again with the object of separating the antioxidant

and vitamin E. With tomatoes, as in the case of lettuce, diphasic separation of the material between petroleum ether and 92% methyl alcohol separated the fractions into a vitamin E portion and an antioxidant portion respectively. However, in the case of wheat germ oil, no clear cut separation of the two fractions could be made by this diphasic separation; in fact, the more active antioxidant fraction was found in the petroleum ether fraction. They concluded that in these natural antioxidant fractions there must be different types of active compounds present. Olcott and Mattill (17) fractionated the unsaponifiable from wheat germ, cottonseed, and palm oils, and concentrated the antioxidant by distillation under high vacuum. Active fractions were obtained but no further identification as to the chemical nature of the fractions was made.

In 1936 the activity of a commercial preparation of lecithin was tested (18) but was found to have very little activity. The activity present was found to be due to cephalin, an impurity, and not to lecithin. The particular portion of the cephalin molecule responsible for its antioxygenic action was probably the monobasic phosphoric acid radical. The tocopherols, α , β , γ , were tested in 1937 (19) and were found to be effective in protecting lard. The degree of protection offered by them was not proportional to the vitamin E activity.

Green and Hilditch in 1937 (20), after digesting Soya bean meal with dilute solutions of organic acids,

extracted with methyl alcohol a viscous gum which possessed marked antioxidant properties. It was similar in properties to those concentrates isolated by Olcott and Mattill (loc. cit.) from the unsaponifiable fractions of wheat germ oil. The yield of the concentrate was many times greater from the extracted oil cake than from the fatty oils themselves.

Olcott and Mattill (21) divided the inhibitors they studied into three groups: - (I) the acid type, (II) inhibitols and hydroquinone, and (III) the phenolic type, including α-napthol, pyrogallol, catechol, and others. The vegetable oils are protected by types (I) and (III) but not by (II); distilled esters of vegetable oils, lard, and esters of lard, and distilled fatty acids are protected by types (II) and (III), but not by (I). A synergistic effect was noted in many cases, which showed that in general, any type (I) inhibitor, used in conjunction with any type (II) or type (III) compound, prolongs the induction period of animal fats and of pure esters to a much greater extent than would be expected from a summation of the effects of each used alone.

Experimental

A. Apparatus

The technique involving the use of the Barcroft-Warburg apparatus as described by Johnston and Frey (17) with slight modifications was used in this work.

As the fatty oils, i.e. olive oil, Soya bean oil, Alfalfa seed oil, etc., presented a rather long induction period, oleic acid was chosen as the test material. Oleic acid was also used because of the fact already cited that natural antioxidant fractions isolated from vegetable oils have no inhibiting effect when replaced in the same or different vegetable oils (12,13). The oleic acid used was purified from crude oleic acid by distillation under reduced pressure of 4 - 5mm. at 205° - 210°C.

The reaction vessels of the Barcroft-Warburg apparatus were placed in a water bath at a temperature of 59.7° ∓ 0.1°C. Glycerol might have been employed, but the temperature used and the volume of glycerol necessary to fill the bath did not warrant its use. The shaking apparatus was not used because the agitation caused hot water to be thrown on the manometers, thus giving changes in readings because of the increase in temperature on parts not actually suspended in the bath.

The determinations were made by pipetting 1 ml. of the oleic acid into the flask proper (Figure V),

not into the central cup. Those fractions to be tested were then added in small quantities, approximately 100 - 150 mgms., either with a dropper or a small spatula, directly to the oleic acid. The flask was thenflushed with a rapid stream of cylinder oxygen at a pressure of 10 - 15 cm. of Brodie solution, with which the manometers were filled. This solution consisted of the following ingredients: 500 ml. of water, 23 gms. of sodium chloride, 5 gms. of sodium glycocholate, and a few drops of alcoholic thymol solution. A pressure of 10,000 mm. of Brodie solution is approximately equivalent to 760 mm. of mercury. The manometers were set at 29 cm. and equilibrated for 15 minutes. The system was then closed and pressure readings made every 15 minutes, or at other definite intervals. In cases where the oxidation proceeded rapidly to a point where the manometer could no longer be read, the manometer was reset so that the oxidation would continue for the same length of time as in the adjoining determinations. In cases where the oleic acid blank had to be reset several times, readings up to 380 were made and then this manometer was opened and the measurement stopped. Where active isolated fractions were being measured, in which the activity was so great as to give little or no oxidation, readings were made for 280 minutes and then stopped.

Since the Barcroft-Warburg apparatus employs a manometric measurement, it was necessary to account for changes in temperature on the parts of the manometers which are not controlled by suspension in the constant temperature bath. Therefore, with each determination an empty manometer was used as a thermobarameter; any changes in surrounding temperature recorded a change in the pressure and the manometers containing reacting materials could be thus corrected for these changes. The measurements may be converted by converting the pressure readings to cubic millimeters or the results may be interpreted directly from the pressure measurements. The latter method was used in this work as the determinations were relative.

B. Materials used

Three vegetable oils were used, namely; Alfalfa seed oil, Sesame oil, and Soya bean oil. Most of the fractionation work was done on the Soya bean oil as sufficient quantities of the others were unavailable.

Three pure sterols and a hydrocarbon from Alfalfa seed oil were tested for antioxidant activity. These sterols were α-spinasterol, β-spinasterol, δ-spinasterol. The hydrocarbon was not identified. These compounds were isolated and furnished by Mr. L. Carroll King. In addition to the pure sterols a mixture of crude Soya bean sterols was tested.

C. Isolation procedures

In previous experiments, other workers found that the antioxidant fractions were found in the unsaponifiable fractions of the various vegetable oils along with the sterols (6). Since sterols can also be removed directly from the oils by alcoholic extraction, it was thought that this method might also be applied to the isolation of the anti-oxidant fraction.

Equal volmes of Soya bean oil and 95% ethyl alcohol were refluxed for 2-3 hours and then allowed to cool overnight. The alcohol layer at the top was siphoned off, and the alcohol removed by distillation leaving a brownish, viscous oil. This oil was then dissolved in ethyl ether and the ethereal solution washed three times with a solution of sodium bicarbonate and then washed with water until neutral to litmus paper. In this procedure, a persistent emulsion was invariably formed which was finally broken by the use of 95% ethyl alcohol and sodium chloride. The ether was then removed from the oil on a steam beth leaving a fatty acid free residue.

The residue obtained was then partitioned between a petroleum distillate (Skellysolve B) and 90% methyl alcohol. Here again an emulsion formed but it broke readily when allowed to stand about an hour. The epiphasic layer was washed three times with methyl alcohol and the hypophasic layer three

times with Skellysolve B and the respective washings combined with the original solutions. In both cases the solvent was removed on the steam bath. The petroleum distillate layer yielded an oil even more viscous than the original alcoholic extract, while the methyl alcohol layer yielded a gummy-like solid. These two fractions are hereafter referred to as the "petroleum ether fraction" and the "methyl alcohol fraction".

Both the petroleum ether fraction and the methyl alcohol fraction were then separated into a sterol fraction and a non-sterol fraction. This separation was accomplished by the addition of 200mg. of digitonin, dissolved in ethyl alcohol, to 200mg. of the fraction also dissolved in ethyl alcohol. The resulting solution was allowed to stand 3-4 hours until the sterols were completely precipitated.

The sterols, precipitated as digitonides, were removed by centrifugation until the supernatant alcoholic solution was clear. The digitonides were decomposed according to Schönheimer and Dam (18) by dissolving them in pyridine and precipitating the digitonin with ethyl ether. Ten times the volume of pyridine was used. The digitonin was removed by centrifugation and the sterols recovered by evaporation of the pyridine and ether on the steam bath.

The alcoholic supernatant liquid was evaporated on the steam bath and a viscous oil, much lighter in color than the original, resulted. In both cases part of the oil was used for antioxidant determination. The remainder of the oil was redissolved in alcohol and water was added until a permanent cloudiness developed. It was necessary to centrifuge these cloudy solutions to obtain an epiphasic layer and a hypophasic layer. The latter consisted of a brown oil, in both cases, and has been labeled "PENS 1" and "MANS 1", denoting that from the petroleum ether non-sterol fraction and the methyl alcohol non-sterol fraction respectively. Water was added in small amounts to the epiphasic layer and the centrifugation repeated, until no more brown oil could be separated. The alcohol and water were evaporated from the epiphasic layer leaving gummy-like residues labeled "PENS 2" and "MANS 2", denoting that from the petroleum ether non-sterol fraction and the methyl alcohol non-sterol fraction respectively.

Alcoholic extracts were also made of Alfalfa seed oil and Sesame oil but lack of materials prevented further fractionation of the extracts. In the case of Alfalfa seed oil when the alcoholic extract residue was dried, a portion, insoluble in ethyl ether, was found. It contained a large quantity

of phosphorus and therefore was thought to be a phosphatide.

Determinations of antioxidant activity were made on all the fractions: fatty acid free alcoholic extracts, the petroleum ether fraction, the methyl alcohol fraction, the sterol and the non-sterol fractions from both the petroleum ether and methyl alcohol fractions, and the fractions labeled "PENS 1," "PENS 2", "MANS 1", and "MANS 2". A test was also run on the phosphorus containing fraction. The results with manometer readings corrected are recorded in Tables I-VI and also plotted on Figures I-IV.

Table I

Alcohol Extracts

	Corrected Manometer Readings*		
Time-Minutes	Soya Bean Oil	Sesame Oil	Alfalfa Seed Oil
15	-6	-12	3
30	3	-11	9
45	14	-13	16
60	22	-15	
75	29	-17	
90	36	-17	
105	39	-17	46
120	44	-18	51
135	48	-18	
150	48	-16	
165	52	-16	92
180	56	-17	
195	68	- 9	
210	76	- 7	
225	84	- 5	
240	83	- 3	
255	84	0	
270	89	2	
285	89	2	

* Corrected for temperature and pressure

Table II

Alcoholic Extract of Soya bean oil

Petroleum Ether and Methyl Alcohol Fractions

	Corrected Manometer Readings*	
Time-Minutes	Petroleum Ether Fraction	Methyl Alcohol Fraction
15	0	0
30	-6	1
45	-6	6
60	-6	11
75	-9	16
90	-9	20
105	-7	31
120	-7	35
135	-5	41
150	-9	47
165	-7	52
180	-6	57
195	-5	62
210	-4	65
225	-4	65
240	-3	
255		
270		
285		89

* Corrected for temperature and pressure

Table III

Alcoholic Extract of Soya Bean Oil
Non-sterol Fractions

Corrected Manometer Readings*

Time-Minutes	PENS**	MANS***
15	-13	10
30	-26	22
45	-31	42
60	-37	58
75	-39	80
90	-43	99
105	-49	122
120	-50	145
135	-55	176
150	-51	192
165	-51	221
180	-54	245
195	-57	271
210	-56	302
270	-56	

* Corrected for temperature and pressure

Table IV

Alcoholic Extract of Soya bean oil
Alcohol-Water Separation of Non-sterol Fractions

Corrected Manometer Readings*

Time-Minutes	Oleic acid**	PENS 1	PENS 2	MANS 1	MANS 2
15	35	-14	-14	-5	-2
30	78	-22	-11	1	17
45	115	-28	-7	5	33
60	160	-31	4	14	66
75	208	-33	18	25	101
90	251	-33	30	37	139
105	300	-31	43	49	181
120	350	-29	56	64	216
135	400	-29	70	78	259
150		-27	82	92	298
165		-24	95		340
180		-22	108		383
195		-21	120		422
210		-21	126		
225		-18	134		
240		-16	162		
255		-13			
270		-10			

* Corrected for temperature and pressure

** Blank run

Table V

Sterols and Hydrocarbon

Corrected Manometer Readings*

Time	α-Spinasterol	β-Spinasterol	δ-Spinasterol	Crude Soya bean sterols	Hydrocarbon	M.A.** sterols	P.E.*** sterols	**** Oleic acid
15	29	35	30	31	31	22	23	28
30	64	75	66	74	69	65	66	64
45	98	113	104	110	105	105	103	98
60	150	157	154	156	154	137	140	154
75	175	184	182	183	182	179	181	182
90	211	224	220	222	220	215	217	220
105	268	273	271	271	269	257	262	271
120						295	234	

* Corrected for temperature and pressure

** Methyl Alcohol fraction

*** Petroleum Ether fraction

**** B lank run

Table VI

Blank Runs on Oleic Acid

Corrected Manometer Readings*

	RUN NUMBERS						
Time-Minutes	1.	2.	3.	4.	5.	6.	7.
15	22	20	19	21	20	17	16
30	46	48	44	47	42	39	46
45	70	69	69	72	64	61	68
60	92	95	95	100	101	96	98
75	120	123	123	130	127	126	126
90	145	149	153	165	143	147	153
105	180	182	175	188	174	178	185
120	201	199	206	201	195	202	206
135	226	224	230	231	221		
150	250	253	258	263	247		
165	295	298	301	304	299		

* Corrected for temperature and pressure

Figure I

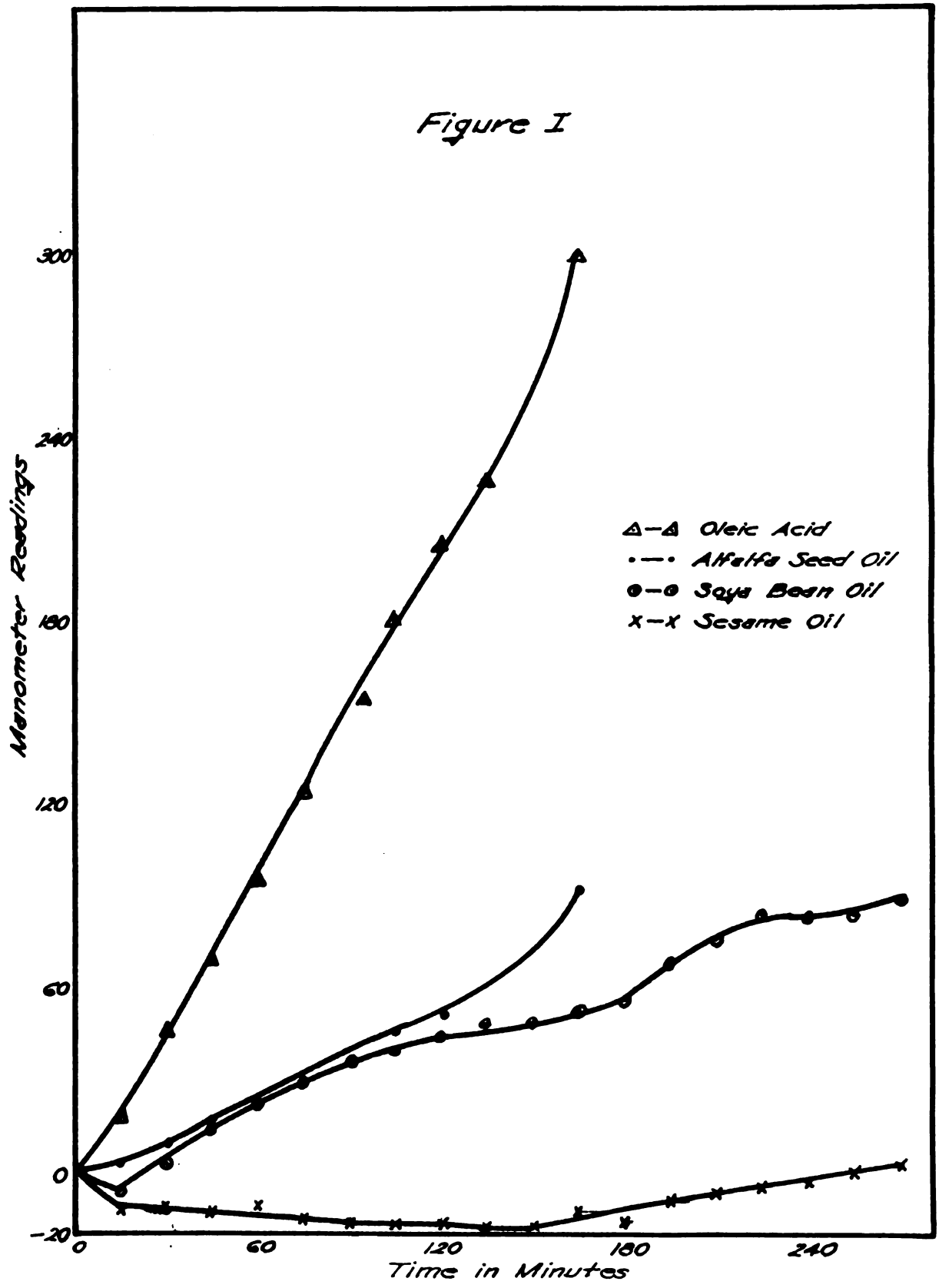
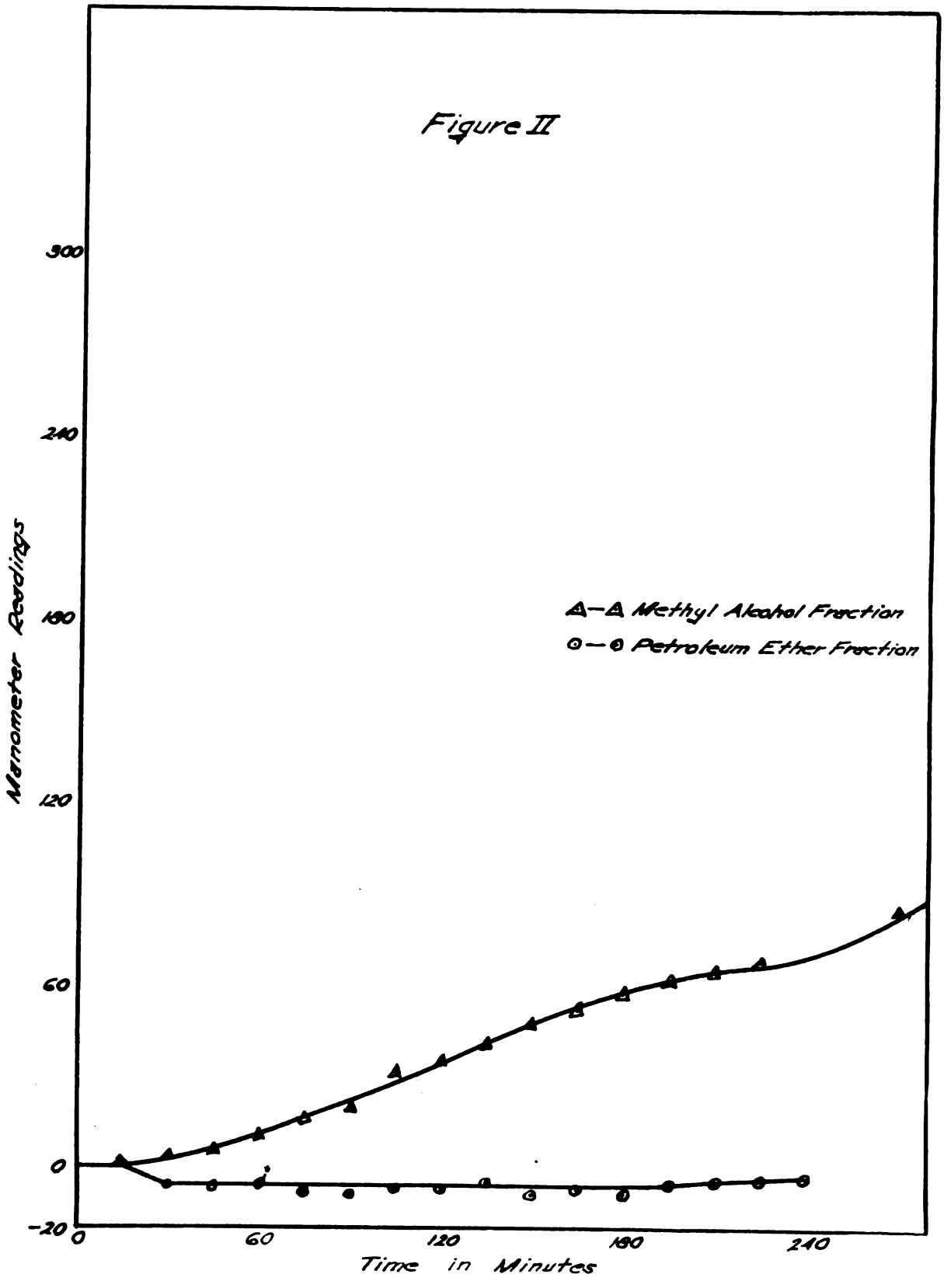

300
240
180
120
60
0
-20
Manometer Readings
0
60
120
180
240
Time in Minutes
Δ—Δ Oleic Acid
•—• Alfalfa Seed Oil
●—● Soya Bean Oil
x—x Sesame Oil

Figure II


300
240
180
120
60
0
-20
Manometer Readings
0
60
120
180
240
Time in Minutes
Δ—Δ Methyl Alcohol Fraction
⊙—⊙ Petroleum Ether Fraction

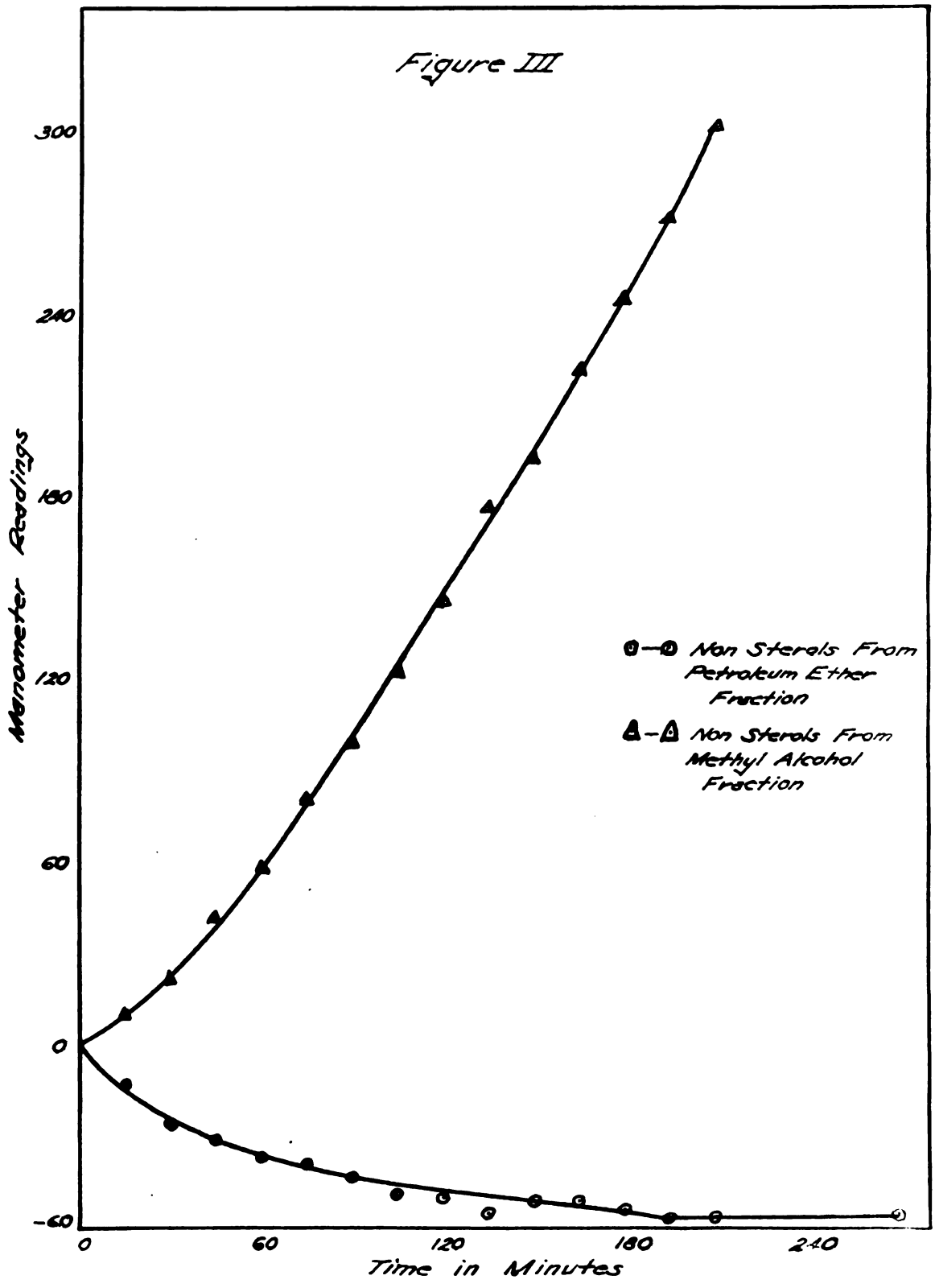


Figure III
300
240
180
120
60
0
-60
Manometer Readings
0
60
120
180
240
Time in Minutes
O—O Non Sterols From Petroleum Ether Fraction
Δ—Δ Non Sterols From Methyl Alcohol Fraction

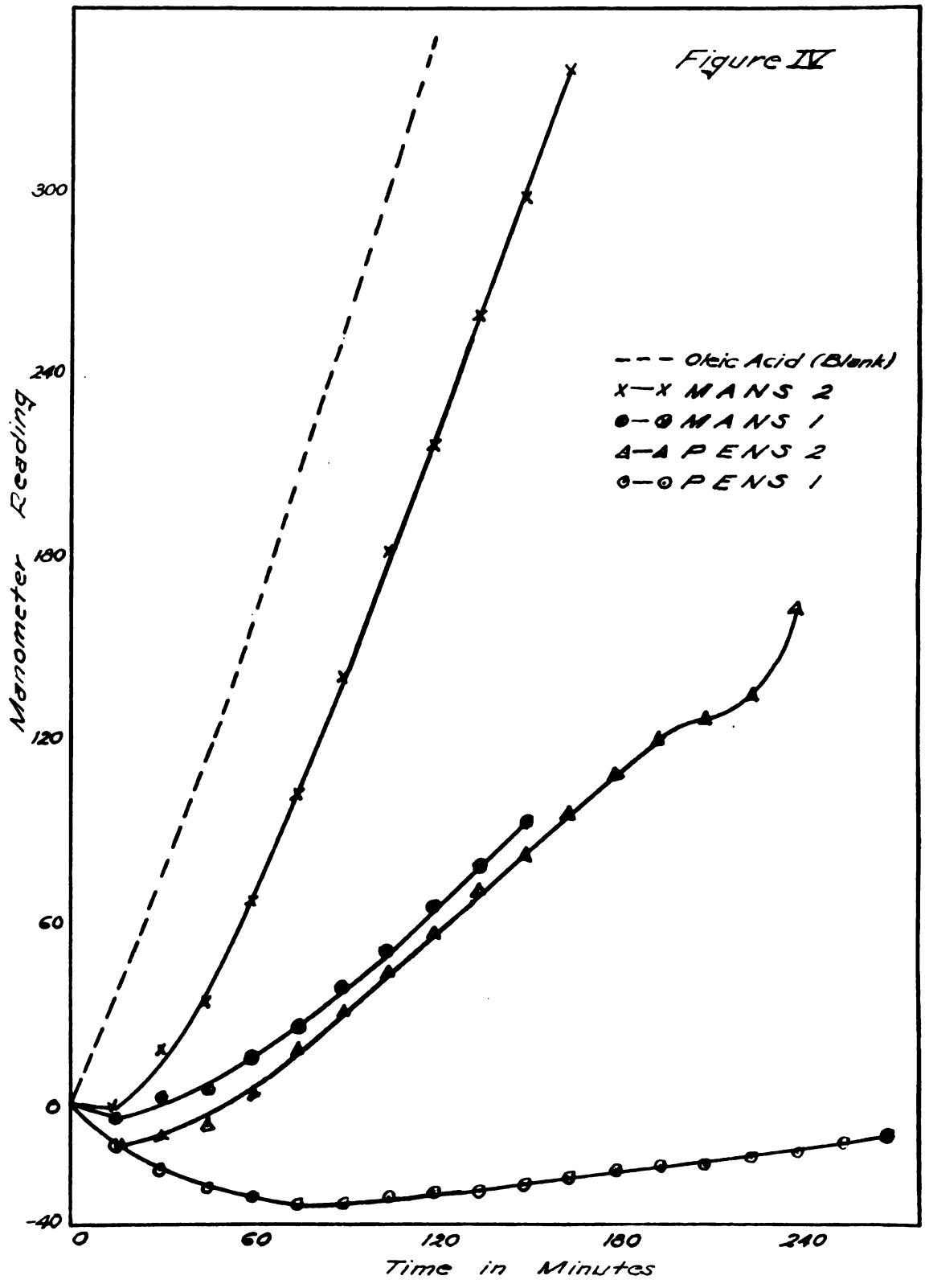
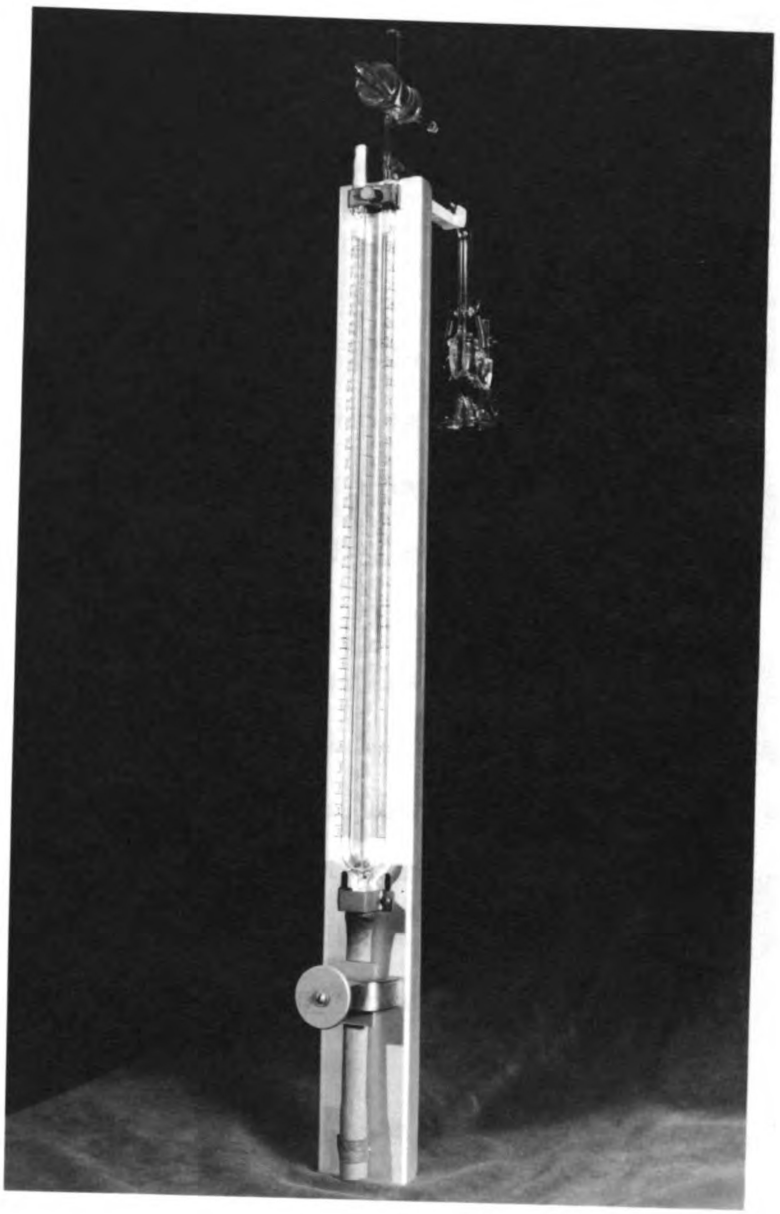


Figure IV
300
240
180
120
60
0
-40
Manometer Reading
0
60
120
180
240
Time in Minutes
--- Oleic Acid (Blank)
x—x MANS 2
●—● MANS 1
Δ—Δ PENS 2
○—○ PENS 1

Figure V



Manometer with Reaction Flask

Discussion

The alcoholic extracts of Sesame oil, Soya bean oil, and Alfalfa seed oil showed marked antioxidant activity in inhibiting the oxidation of oleic acid. The alcoholic extraction of the vegetable oils, therefore, is applicable to the isolation of antioxidant fractions from these oils.

The partitioning of the alcoholic extract of Soya bean oil between a petroleum distillate and 90% methyl alcohol resulted in a higher concentration of the antioxidant fraction in the petroleum distillate layer than in the methyl alcohol layer, although the latter still retained some activity.

The fractionation into sterols and non-sterols of both the petroleum ether and methyl alcohol fractions confirmed the results of others that in the case of the sterol fractions,there is no antioxidant activity. With the non-sterols, the petroleum ether fraction showed very marked activity, while the methyl alcohol fraction showed but very little activity.

The pure sterols and the crude Soya bean sterols showed no activity whatsoever, a fact giving further confirmation of the inactivity of sterols as antioxidants. The hydrocarbon was also inactive.

The fractions "PENS 1", "PENS 2", and "MANS 1", "MANS 2" show the further concentration of the antioxidant in the petroleum ether. While fractions "PENS 2" and "MANS 1"

were moderately active, "MANS 2" was practically inactive, but "PENS 1" was by far the most active concentrate isolated.

Negative readings of pressure were obtained in several cases in testing isolated fractions, i.e., an indication that the material was giving off a volatile substance. The most probable explanation is that not all the solvent had been removed from the fraction being tested. Although all the fractions were dried in a vacuum oven from 4-8 hours, it appears that some of the solvent was still present in the material (22). In all cases where negative readings were obtained an equilibrium was eventually established and in some cases oxidation proceeded from the equilibrium point.

It is suggested that this method of alcoholic extraction of vegetable oils may have commercial application in that the sterols and an antioxidant fraction can be isolated and the oil itself recovered without appreciable loss or damage to other properties of the oils. The sterols and antioxidants could then be separated and used.

Summary and Conclusions

1. The use of the Barcroft-Warburg apparatus for the activity of antioxidants, in relation to fatty oils, is precise and convenient.
2. The alcoholic extraction of vegetable oils as applied to the isolation of sterols can also be applied to the natural antioxidants present in such oils.
3. Soya bean oil, Sesame oil, and Alfalfa seed oil contain antioxidants which can be isolated by this alcoholic extraction.
4. The antioxidant material from the alcoholic extract can be concentrated in the petroleum distillate layer when the material is partitioned between the petroleum distillate and 90% methyl alcohol.
5. The petroleum distillate fraction can be further separated into an active non-sterol fraction, and a completely inactive sterol fraction.
6. The non-sterol fraction of the material from the petroleum distillate fraction contains a brown oil, the most active material isolated. This oil separated from an alcoholic solution of the petroleum ether non-sterol fraction upon the addition of water. Centrifugation is necessary to separate the brown oil from the alcohol-water mixture.

Bibliography

1. Moureu, C. and Dufraisse, C. Compt. rend. Soc. biol., 1922, lxxxvi, 321; Compt. rend. Acad., 1922 clxxiv, 258.

2. Anderegg, L. T. and Nelson, V. E. Milk powder as food. II Observations on the existance of vitamin E. Ind. Eng. Chem. 18:620 (1926).

3. Mattill, H. A. The oxidative destruction of vitamins A and E and the protective action of certain vegetable oils. J. Am. Med. Assoc. 89:1505 (1927).

4. Cummings, M. J. and Mattill, H. A. The autoxidation of fats with reference to their destructive effect on vitamin E. J. Nutrition 3:421 (1931).

5. Olcovich, H. S. and Mattill, H. A. The unsaponifiable lipids of lettuce. I Carotene. J. Biol. Chem. 91:105 (1931).

6. Mattill, H. A. and Crawford, Blanche. Autoxidation of corn oil as related to its unsaponifiable constitituents. Ind. Eng. Chem. 22:341 (1930).

7. Mattill, H. A. Antioxidants and the autoxidation of fats. J. Biol. Chem. 90:141 (1931).

8. Olcovich, H. S. and Mattill, H. A. Isolation of the natural antioxidant of lettuce. J. Biol. Chem. 92:xxxi (1931).

9. Olcott, H. S. and Mattill, H. A. Unsaponifiable lipids of lettuce. II Fractionation. J. Biol. Chem. 93:59 (1931).

10. Olcott, H. S. and Mattill, H. A. The unsaponifiable lipids of lettuce. III Antioxidants. J. Biol. Chem. 93:65 (1931).

11. Royce, H. D. and Lindsey, F. A. Jr. Ind. Eng. Chem. 25:1047 (1933).

12. Adams, R. and Baker, B. R. Structure of Gossypol. XXV Synsthesis of desapogossypolone tetramethyl ether. J. Am. Chem. Soc. 63:535 (1941).

13. Olcott, H. S. Unpublished observations. Cited in Chem. Rev. 29:257 (1941).

14. Boeseken, J. Coden, W. D. and Kip, C. J. Rec. trav. chem. 55:815 (1936).

15. Hilditch, T. P. and Sleightholme, J. J. Studies on the nature of antioxygens present in natural fats. I Separation of fatty derivatives from antioxygens by distillation. J. Soc. Chem. Ind. 51:39t (1932).

16. Bradway, E. M. and Mattill, H. A. The association of fat-soluble vitamins and antioxidants in some plant tissue. J. Am. Chem. Soc. 56:2405 (1934).

17. Olcott, H. S. and Mattill, H. A. Antioxidants and autoxidation of fats. VI Inhibitols. J. Am. Chem. Soc. 58:1627 (1936).

18. Olcott, H. S. and Mattill, H. A. Antioxidants and autoxidation of fats. IV Lecithin as an antioxidant. Oil and Soap 13:98 (1936).

19. Olcott, H. S. and Emerson, O. H. Antioxidants and autoxidation of fats. IX The antioxidant properties of the tocopherols. J. Am. Chem. Soc. 59:1008 (1937).

20. Green, T. G. and Hilditch, T. P. Studies on the nature of antioxygens in natural fats. III The occurence of antioxygenic compounds in extracted Soya bean oilcake. J. Soc. Chem. Ind. 56:23t (1937).

21. Olcott, H. S. and Mattill, H. A. Antioxidants and autoxidation of fats. VII Preliminary classification of inhibitors. J. Am. Chem. Soc. 58:2204 (1936).

22. King, L. C. The unsaponifiable fraction of Alfalfa seed oil. Masters Thesis M. S. C. (1938).

OCT 27'58

OCT 25 1976

T547 K51 143862
Kindle

MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 02446 7379