TABLE 5

Calculated yields of normal oleic acid, of various degrees of purity, obtainable from cottonseed and peanut oil hydrogenated under different conditions. (On basis of complete separation of solid and liquid acids, impurity consisting of linoleic acid only.)

Run No.	Kind of oil	Hyd. time, min. to crit. I.V.	Percentage composition of fatty acids at their critical I.V.*				Per cent yield of normal oleic acid		
			Sat.	Iso-oleic	Normal oleic	Lin-oleic	95% pure	90% pure	85% pure
CO-23	Cotton	190	31.0	20.4	44.6	4.0	45	48	50
CO-11	Cotton	101	31.7	15.8	47.7	4.8	48	52	54
CO-18	Cotton	19	32.5	14.3	47.6	5.6	40	52	54
CO-19	Cotton	67	32.8	13.2	48.1	5.9	40	53	55
CO-15	Cotton	24	32.8	12.4	48.9	5.9	40	53	56
CO-12	Cotton	31	34.1	11.2	47.5	7.2	<40	49	56
CO-9	Cotton	12	34.2	10.7	47.8	7.3	<40	47	55
CO-16	Cotton	98	35.1	11.0	45.7	8.2	<40	40	53
CO-17	Cotton	21	35.2	10.2	46.2	8.4	<40	40	53
CO-20	Cotton	72	39.6	8.5	39.3	12.6	<40	<40	<40
PO-23	Peanut	135	21.4	14.0	62.9	1.7	66	68	70
PO-12	Peanut	14	25.7	7.1	61.3	5.9	58	68	70
PO-20	Peanut	30	30.0	4.8	55.0	10.2	45	59	64

* For cottonseed oil, 65.7; for peanut oil, 72.6.

purity, 53 per cent of oleic acid of 90 per cent purity, and 48 per cent of oleic acid of 95 per cent purity; and from peanut oil, 70 per cent of oleic acid of 85 per cent purity, 68 per cent of oleic acid of 90 per cent purity, and 66 per cent of oleic acid of 95 per cent purity.

Acknowledgment

The authors wish to acknowledge the courtesy of Mr. A. R. Diehl, of the Cudahy Packing Company, Memphis, Tennessee, in furnishing the catalyst used for a large part of this work; they are indebted also to Messrs. G. E. Mann, W. G. Bickford, and C. L. Hoffpauir for valuable suggestions and assistance in carrying out the analytical phases of the investigation.

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Factors Affecting the Stability of Cottonseed Oil. A Study of the Antioxygenic Activity of Alpha-Tocopherol[1]

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The report of the isolation of the tocopherols from vegetable oils (1) was followed closely by a description of their antioxygenic activity (2). Earlier studies of the sterol-free unsaponifiable fractions (inhibitols) of vegetable oils containing tocopherols formed a valuable background for the investigations of the antioxygenic activity of the tocopherols. The tocopherols are considered to contribute some if not most of the antioxygenic activity of the inhibitols, although it is recognized that other antioxidants may be isolated (3) (3a).

The mechanism by which the tocopherols inhibit oxidation is only partly understood; this is to be expected since the autoxidative reactions which they inhibit are complex and still obscure. In previous investigations of natural antioxidants several interesting observations have been made for which the antioxygenic activity of the tocopherols may be wholly or partly responsible. In an investigation of the inhibitols their effectiveness was found to be approximately proportional to the amounts used (4), but they were ineffective when added to the oils from which they were obtained (5). A completely satisfactory explanation of this phenomenon has not been advanced, but it has been suggested that the amounts of inhibitols added to the original oils were too small (6) and that other substances may have prevented the functioning of the inhibitols (3). In another investigation adequate explanations were lacking for the unusual behavior of a concentrate of the unsaponifiable substances of refined prime summer yellow cottonseed oil obtained in the most volatile fraction of molecularly distilled oil (7). The fraction was shown to contain the bulk of the protective substances of the oil, but it rapidly accumulated peroxides during air-blowing

[1] Agricultural Chemical Research Division Contribution No. 84.

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stability tests (Fig. 6). The present investigation was undertaken in recognition of the need for further knowledge of the antioxidant activity and reaction mechanism of the tocopherols.

Experimental

The preparation of purified materials for use in this investigation was an important part of it. In an effort to facilitate the interpretation of results and to assure their validity, care was taken to secure maximum protection against oxidative deterioration, the action of light and contamination during the preparation of materials. The materials were used as soon as possible

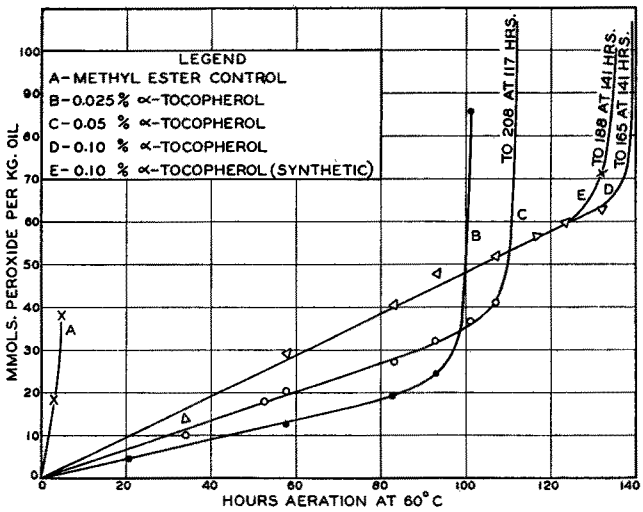

FIG. 1

after their preparation; all experiments were repeated using freshly prepared materials.

Stability tests were conducted under conditions similar to those of the Swift test except that samples were aerated with 13 cc. of air per minute at 60° C., in the absence of light.

Preparation of Substrates. The methyl esters of cottonseed oil were prepared from a commercially refined prime summer yellow grade [3] of oil by refluxing for 40 minutes in a nitrogen atmosphere, 1 kg. of the oil in 2 liters of a 0.065 N solution of sodium methylate in absolute methyl alcohol. The methyl esters, after extraction, washing and removal of solvent, were distilled (boiling range 125-130° C. at 0.05 to 0.1 mm. pressure) from the unsaponifiable constituents of the oil. This preparation is similar to one previously described (5) except that in the earlier work dry HCl gas was used as catalyst. The distilled esters had the following characteristics: Wijs iodine number, 111.0; % free fatty acids as oleic, 0.3%; peroxide value, 0.0.

Methyl linoleate was prepared by the debromination of crystalline tetrabromostearic acid (m.p. 115.3° C.), using a procedure previously described (8). The final product was purified by distillation (boiling range 125-128° C. at 0.05-0.1 mm. pressure). It had a Wijs iodine number of 173.4 (theory requires 172.4) and a peroxide value, 2.5.

Methyl oleate was prepared by low-temperature crystallization of U. S. P. olive oil according to a method previously reported (9). The specimen had the following characteristics: Wijs iodine number, 85.3 (theory requires 85.6); $n\frac{25}{D} = 1.4500$; peroxide value, 0.0.

Tocopherol Samples. Natural α-tocopherol allophanate (m.p. 158.5°-159.5° C.) was hydrolyzed with a 4% solution of KOH in absolute methyl alcohol in a nitrogen atmosphere to yield golden colored α-tocopherol. Synthetic α-tocopherol [4] was used in corroborative experiments. A sample of synthetic γ-tocopherol [4] was also used for comparison. α-tocopherylquinone was prepared by oxidizing natural α-tocopherol with silver nitrate according to a method previously reported (10). The final product contained 0.3% α-tocopherol.

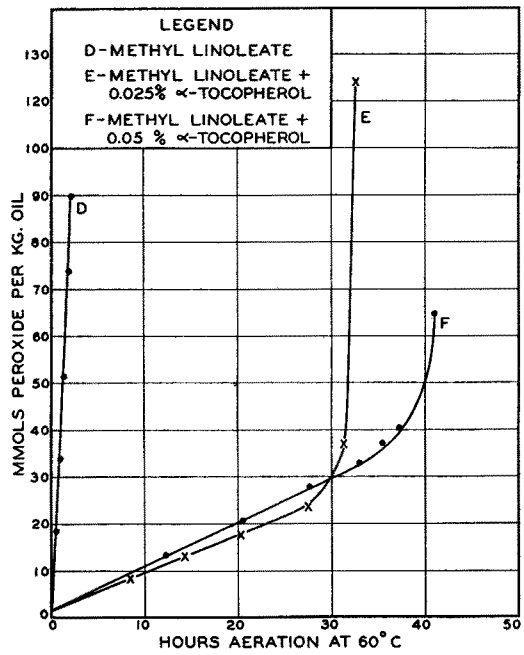

FIG. 2

Cephalin fraction. The crude phosphatides of 1 kg. of prime crude cottonseed oil were extracted with five 1-liter portions of ethyl alcohol. The cephalin fraction was purified by dissolving the crude phosphatides in peroxide-free ether and precipitating them from acetone; the fraction was reprecipitated five times, using acetone and alcohol, alternately, as the precipitants, acetone being the final precipitant.

Tocopherol estimations were made by the Emmerie Method (11) using a neutral-wedge spectrophotometer (12) for measurements of color intensity.

The potency of α-tocopherol was determined at several levels in the methyl esters of cottonseed oil (Figure 1). The peroxide-accumulation curves show that 0.025% was approximately 60%, and 0.05% was approximately 80%, as effective as 0.1% α-tocopherol. Similar observations were made with samples containing γ-tocopherol which was approximately twice as effective as α-tocopherol; 0.025% γ-tocopherol (195 hours) was approximately 90% as effective as 0.05% γ-tocopherol (210 hours). The peroxide-accumulation curves showing the activity of α-tocopherol added to pure methyl linoleate indicate that 0.025% was approximately 75% as effective as 0.05% (Figure 2);

[3] Made available through the courtesy of the Barrow-Agee Laboratories, Memphis, Tenn.

[4] Generously donated by Merck & Co., Inc., Rahway, N. J.

no appreciable difference was observed in the stability of methyl oleate samples containing 0.025% and 0.05% α-tocopherol (Figure 3). The addition of 0.2% α-tocopherol to refined prime summer yellow cottonseed oil did not appreciably affect the stability of the oil (Figure 5); this observation was similar to those previously reported in studies of the antioxygenic activity of the inhibitols and tocopherols in which it was found that the inhibitols (5) and 0.01% and 0.02% α-tocopherol (2) were inactive in cottonseed oil and in the crude esters of hydrogenated cottonseed oil, respectively. The results indicate that the tocopherols function most efficiently at lower levels and with decreasing efficiency at each successively higher level.

The effect of unsaturation is apparent on comparing the stability of the methyl oleate substrate and α-tocopherol-stabilized methyl oleate with that of methyl linoleate substrate and α-tocopherol-stabilized methyl linoleate (Figures 2 and 3). The samples of methyl oleate to which 0.025% and 0.05% α-tocopherol was added were approximately ten times as stable as methyl linoleate to which the same proportions of α-tocopherol had been added. The methyl oleate substrate appeared to have an induction period, although the duration could not be determined exactly; an induction period was not detected in the methyl linoleate substrate. The development of tallowy and rancid-pungent odors in the methyl oleate samples and the α-tocopherol-stabilized methyl linoleate samples, respectively, was observed to occur at the same time as the acceleration of the peroxide formation (at the end of the induction period).

It has been reported that the inhibitols delay the accumulation of peroxides and are destroyed in a rapidly oxidizing substrate; also that there is evidence of the existence of two kinds of peroxides, since the inhibitols did not completely remove the peroxides when they were added to oxidizing fats (4). These observations, in suggesting that the reactivity of the tocopherols is predominantly toward the active form of peroxides, are closely related to those of the present investigation. As shown in Figures 1, 2, and 3, the rate of peroxide-accumulation, as well as the stability of the samples, was observed to increase upon the addition of successively higher amounts of α-tocopherol and, since the tocopherol was presumably oxidized in reacting with the least stable peroxides, it appears that those peroxides which accumulated were of the more stable type. Corroborative evidence that the rate of peroxide accumulation is related to the tocopherol content was supplied in the results of an experiment in which 0.2% α-tocopherol was added to refined prime

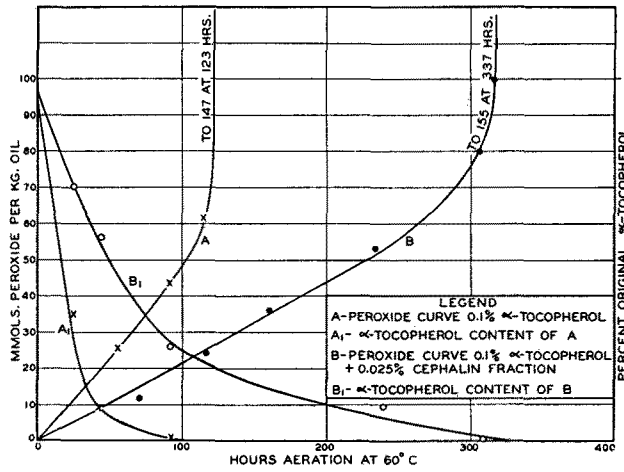

FIG. 4

summer yellow cottonseed oil originally containing 0.1% (α plus γ) tocopherols. An appreciable increase in the rate of peroxide accumulation was observed (Figure 5), although, as indicated, the additional tocopherol was ineffective in appreciably increasing the stability of the oil. The means of definitely establishing a relationship between the decrease in efficiency of action of the tocopherol at each successively higher level of concentration and the rate and extent

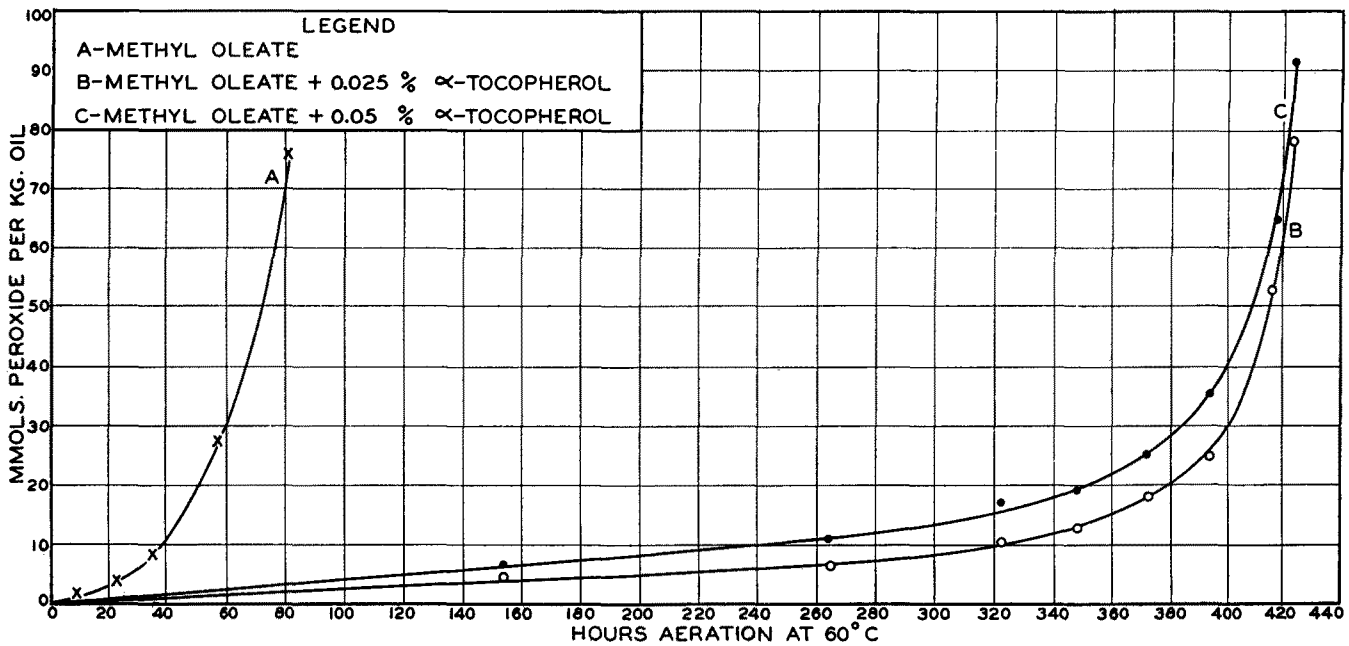

FIG. 3

of peroxide accumulation at the respective levels has not been realized experimentally.

The results show that the oxidation of α-tocopherol in the methyl esters of cottonseed oil did not proceed at a uniform rate, but that the initial rate of oxidation was quite rapid (Figure 4). Similar results were obtained in a study of the rate of oxidation of α-tocopherol (6) (14). α-Tocopherylquinone (0.1%), another oxidation product, was shown to be inactive as an antioxidant in lard (15); the inactive samples contained 0.0% and 4% α-tocopherol. In the present work

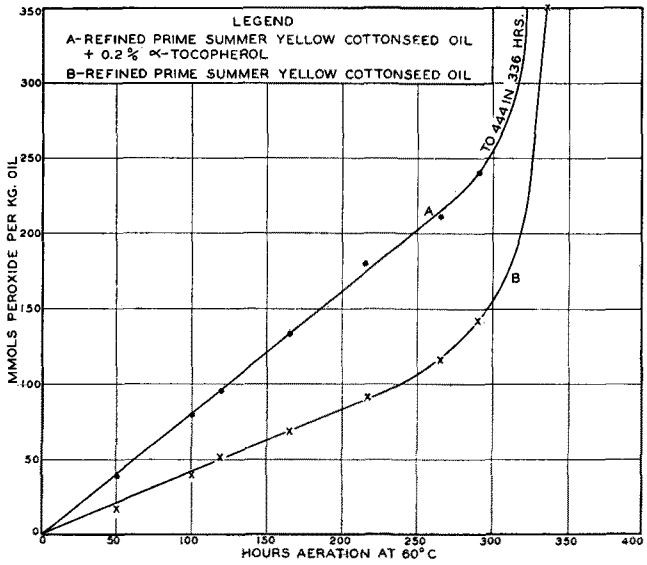

FIG. 5

pherol in lard (13); in the latter work it was found that 93.4% of the original α-tocopherol content was oxidized in approximately the first half of the induction period. In order to determine the rate of oxidation of tocopherol in a saturated ester, 0.1% was added to ethyl laurate (I.V. 0.0) and the sample was aerated. The results of tocopherol estimations showed that the oxidation of the tocopherol proceeded slowly and, therefore, that the rapid initial rate of oxidation of α-tocopherol in stabilizing the methyl esters of cottonseed oil was evidently caused by substances present or developed in the unsaturated esters.

Among the most important factors affecting the antioxygenic action of the tocopherols in natural oil is the influence of other minor constituents. One of these substances, the cephalin fraction of the phosphatides, has been shown to co-act with the tocopherols in a most remarkable manner. This co-action, or synergism, is an example of an "acid-type" substance acting synergistically with a substance possessing the properties of the "phenolic" type (3) (5). The enhancing effect of the addition of 0.025% of the cephalin fraction of crude cottonseed oil phosphatides increased the potency of 0.1% α-tocopherol approximately three times (Figure 4). An indication of the mechanism is shown in the retarded rate of oxidation of the tocopherol. The results of an experiment in which the cephalin fraction content was varied (Table 1) indicate an effect of promoter action; the smallest quantity tested, 2.5 p.p.m., was approximately two-thirds as effective as 250 p.p.m.

Recently the mechanism of the synergistic activity of the tocopherols and phosphoric acid has been sufficiently investigated to suggest that phosphoric acid favors ring closure in one of the oxidation products of tocopherol, toco(hydro)quinone, to reform tocopherol (6) (14). α-Tocopherylquinone (0.1%), another oxidation product, was shown to be inactive as an antioxidant in lard (15); the inactive samples contained 0.0% and 4% α-tocopherol. In the present work

TABLE 1

Effect of the Cephalin Fraction of the Phosphatides of Cottonseed Oil on Methyl Esters Containing α-Tocopherol

% α-Tocopherol	Cephalin Fraction	Stability [1]
	p.p.m.	*hrs.*
0.025	250	340
0.025	125	330
0.025	25	295
0.025	12.5	250
0.025	2.5	225
0.025	0	85
0	250	10
0	0	5

[1] Air-blowing accelerated method 60°C.

0.15% α-tocopherylquinone stabilized the methyl esters of cottonseed oil slightly (30 hours, control 5 hours), but was quite active when used with 0.025% cephalin (10 hours); the combination stabilized the methyl esters for 175 hours. During the induction period the tocopherol content was at most very low. The sample of α-tocopherylquinone used in this experiment contained some 2,7,8-trimethyl-2-phytyl-5,6-chromanedi-

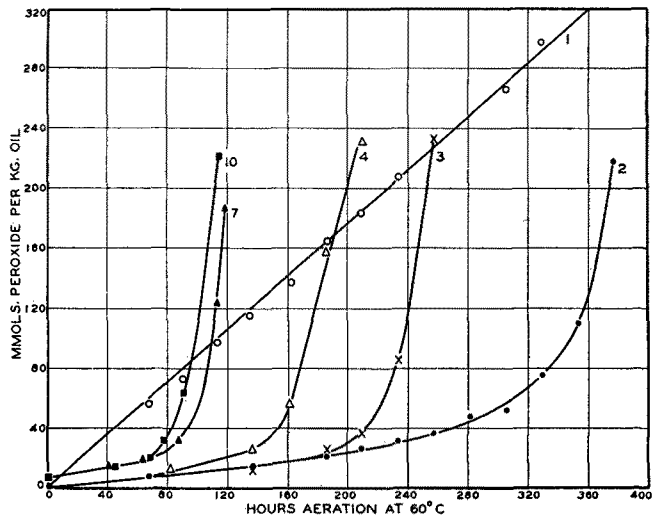

FIG. 6. Peroxide-accumulation curves of fractions of molecularly distilled cottonseed oil; curve numbers correspond to the fraction numbers of the distilled portions; curve number 1 represents the peroxide accumulation of the first, most volatile fraction; graph reproduced (7).

one, as shown by its slightly red color, and 0.3% α-tocopherol. The addition of 0.1% of the α-tocopherylquinone to methyl esters containing 0.1% α-tocopherol did not appreciably affect the stability of the samples.

Summary

The results indicate that the tocopherols function most effectively at lower levels of concentration and with decreasing efficiency at higher levels. The data suggest that the reactivity of α-tocopherol is predominantly towards the active peroxides and show that the rate and extent of peroxide accumulation during the induction period is dependent on the tocopherol content.

The initial rate of oxidation of α-tocopherol was found to be very rapid; the addition of small amounts

of the cephalin fraction markedly retarded the rate of oxidation.

The antioxygenic activity of α-tocopherol was greatly increased by the addition of the cephalin fraction; the effect was not proportional to the content of cephalin fraction; instead, an effect of promotor action was observed.

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Effect of Sodium Chloride in Glycerol Analysis by Oxidation

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Introduction

Investigational work instituted in this laboratory has established the fact that C. P. sodium chloride in quantities of 0.1 to 4.0 grams gives an apparent oxidizable content amounting to approximately 0.21 per cent when oxidized by potassium dichromate according to the American Oil Chemists' Society standard method for the determination of glycerol in soap (1). In addition, it was found that as the volume of concentrated sulfuric acid added in the determination was decreased or increased the per cent apparent oxidizable matter correspondingly suffered a diminution or increase. Finally it was shown that as increasing amounts of C. P. glycerol were added to the same weight of the C. P. sodium chloride the per cent apparent oxidizable due to the sodium chloride (after subtracting the per cent oxidizable due to the glycerol) likewise increased.

As a result of the above experimental work it is apparent that to accurately determine the amount of glycerol in salt recovered from soap lyes, pickles, etc., by dichromate oxidation the sodium chloride must first be removed.

TABLE I

Bichromate Oxidation of Sodium Chloride Before and After Decomposition With Concentrated Sulfuric Acid

Experiment No.	Sample	Direct Oxidation of C. P. NaCl	Oxidation of C. P. NaCl after decomposition with concentrated H_2SO_4
	Grams	*Pct. oxidizable as glycerol*	*Pct. oxidizable as glycerol*
1	1.0	0.18	0.05
2	1.0	0.19	0.04
3	1.0	0.20	0.03
4	1.0	0.21	0.03
5	1.0	0.23	0.03
6	1.0	0.24	0.04
7	1.0	0.23	0.03
8	1.0	0.25	0.03
9	1.0	0.16	0.05
10	1.0	0.17	0.04

Experimental

Preliminary experiments with mixtures of C. P. sodium chloride and glycerol showed that extraction with acetone, 50/50 ethyl alcohol-ethyl ether mixture, and anhydrous alcohol resulted in recovery of approximately only 75 per cent of the glycerol added.

Efforts were next directed toward removing the sodium chloride as hydrogen chloride gas by treating with concentrated sulfuric acid. It is an established fact that the presence of sulfates does not interfere in the dichromate oxidation of glycerol. The results obtained are shown in Table I.

The values obtained by oxidation of the salt after decomposition with sulfuric acid demonstrate that the average value of 0.21 per cent apparent oxidizable as glycerol in C. P. sodium chloride is erroneous and that the value is instead close to 0.04 per cent.

The next step was to add C. P. glycerol to C. P. sodium chloride and ascertain the recovery. The results obtained are given in Table II.

The data given in Table II indicates that the method is accurate within experimental limits. Table III gives the results obtained on analysis of three different samples (A, B, C) of salt recovered from soap lye. As can be seen the results obtained for the glycerol content are considerably lower by the new method and presumably much closer to the actual value.

TABLE II

Recovery of Glycerol in Glycerol Salt Mixtures After Decomposition With Concentrated Sulfuric Acid

Experiment No.	Oxidation of C. P. glycerol + 4 grams C. P. NaCl	Oxidation of same amount of C. P. glycerol in the absence of C. P. NaCl	Oxidation of same amount of C. P. glycerol + 4 grams C. P. NaCl after H_2SO_4 decomposition
	Pct. oxidizable as glycerol	*Pct. oxidizable as glycerol*	*Pct. oxidizable as glycerol*
1	1.33	0.85	0.83
2	1.31	0.85	0.89
3	0.61	0.30	0.34
4	0.60	0.30	0.32
5	0.41	0.16	0.18
6	0.40	0.16	0.18

The procedure by which the above results were obtained is given below. The method is general and applicable to any material consisting of large amounts