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

of sodium chloride containing small percentages of glycerol.

Procedure

Weigh approximately 50 grams ± 0.10 gram of the sample and transfer to a 500 ml. volumetric flask. Partially fill the flask with water and shake until the sample is completely dissolved. Dilute the solution to a volume of 500 ml. by the further addition of water. Allow the solution to stand until any insoluble dirt that is present has settled out.

Pipette a 50 ml. aliquot of the clear solution into a 500 ml. tall type beaker and evaporate on the steam bath to a pasty consistency. This operation usually requires about two hours.

Slowly add 30 mls. of concentrated sulfuric acid to the sample, and allow to stand on the steam bath for about one hour after the frothing has completely subsided. The frothing usually ceases in about ten minutes. This operation should be carried out under a hood with a strong draft.

Remove the sample from the steam bath, cool, and proceed to oxidize, or, if more convenient, allow to stand overnight at room temperature before oxidizing. Proceed to oxidize by the method of the American Oil Chemists' Society (1) for the determination of glycerol in soap bearing in mind that the sample already contains sufficient sulfuric acid for the oxidation.

TABLE III

Bichromate Oxidation of Recovered Salt Before and After Decomposition With Concentrated Sulfuric Acid

Sample	Oxidation of salt as received	Oxidation of salt after decomposition with H_2SO_4	Difference
	Pct. oxidizable as glycerol	*Pct. oxidizable as glycerol*	*Pct. oxidizable as glycerol*
A	0.74	0.47	—0.27
A	0.77	0.45	—0.32
B	0.68	0.41	—0.27
B	0.69	0.40	—0.29
C	1.20	0.81	—0.39
C	1.21	0.80	—0.41

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The Antioxygenic Action of Phosphoric Acid in Association With Tocopherols and Hydroquinones*

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The capacity of tocopherols to act as inhibitors of fat autoxidation is greatly enhanced by small amounts of certain acids, both organic and inorganic, which have little or no stabilizing properties in themselves (1, 2, 3, 4).

A kinetic study has been made of the tocopherol-phosphoric acid-lard esters system. The data of Table I show that phosphoric acid retards the oxidation of α-tocopherol and thereby increases the antioxygenic activity of the phenolic constituent. Phosphoric acid likewise augments the stabilizing capacity of hydroquinones.

TABLE I

The Oxidation of α-Tocopherol in the Ethyl Esters of Lard in the Presence and in the Absence of Phosphoric Acid

Time in hours	Amount of Tocopherol in 1 gram Ester	
	(no H_3PO_4) γ	(0.10% H_3PO_4) γ
0	1000	1000
5	66	660
10	trace	
20	0	376
66		trace
68		0
Induction period hours	11	68

A clue to the mechanism of action of phosphoric acid is provided by the observations recorded in Table II. α-Tocoquinone and phosphoric acid, both ineffective by themselves, nevertheless powerfully stabilized the fat substrate when they were used together. This unexpected stability could only mean a regeneration of some tocopherol because tocohydroquinone like its corresponding quinone is ineffective as an inhibitor (5). The presence of tocopherol in the stabilized substrate was confirmed by biological assay. After exposure to oxygen at 75° for at least a day, the stabilized substrate was fed to three vitamin E-deficient female rats in amounts sufficient to provide each animal with at least 4 mgs. of tocopherol; all three animals had litters. The amount of α-tocopherol formed was determined by photometric analysis according to the Emmerie-Engel method (6) (Table III). Evidently the fat itself serves as a reducing agent for the

TABLE II

Antioxygenic Action of Mixtures of Quinones With Phosphoric Acid

Substrate	% Inhibitor Added	Antioxygenic Index [a] at 75°
Ethyl esters of Lard fatty acids	0.20 Phosphoric Acid	1
	0.10 α-Tocoquinone	1
	0.10 α-Tocoquinone + 0.20 Phosphoric Acid	>82[b]
	0.02 Benzoquinone	4
	0.02 Benzoquinone + 0.20 Phosphoric Acid	>110[b]

[a] Ratio of induction period of stabilized ester to that of control.
[b] When discontinued, these samples were still fresh.

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