acetic acid by lyophilization and evacuation over KOH and P_2O_5 as described above, stirred with 10 volumes of 95% ethanol, and gently heated to 45–50°C. to aid in softening the hard mass. A mixture of flocculent and gummy materials remained undissolved. After being held over-night at 4°C., the mixture was filtered at 4°C. and the precipitate was washed with small portions of cold ethanol. Following removal of alcohol, the two fractions were dried *in vacuo* over P_2O_5.

Sphingolipides. The recent discovery of a long-chain base, phytosphingosine, in corn phosphatides (5) was cause for the examination of pea lipides to determine whether sphingolipides are also present in peas. Accordingly the long-chain base nitrogen content of the various pea lipide fractions was determined. These data, included in Tables I and III, indicate that, whereas there is some type of long-chain base present in pea lipides, peas do not constitute a very good source for the preparation of sphingolipides. The nature of the long-chain base present in pea lipides was not determined in the present study.

Summary

Crude pea lipides were prepared by extracting lyophilized raw peas with chloroform-methanol, 2:1, and found to comprise 6% of the dry weight of the peas. The composition of the various fractions of pea lipides was studied by measuring the nitrogen, phosphorus, glycerol, fatty acid, and sugar contents, also by means of paper chromatography following acid hydrolysis. The crude lipides were fractionated with acetone, and the acetone-soluble portion was subjected to countercurrent distribution between n-heptane and 95% methanol. The heptane fraction was found to consist nearly entirely of mixed triglycerides; the methanol fraction was a mixture of triglycerides, phosphatides, sugars, and nitrogenous materials. The acetone-insoluble fraction contained 10% of phosphatidyl inositol and nearly equal amounts of alcohol-soluble and alcohol-insoluble phosphatides.

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Stability of *d*-*a*-Tocopherol Alone, in Solvents, and in Methyl Esters of Fatty Acids[1]

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THE PREVENTION of oxidative spoilage in fats and fatty foods by adding small quantities of antioxidants has been widely studied during the past 20 years. Tocopherol, the most important naturally-occurring fat stabilizer, apparently interrupts the oxidation reaction as a free radical acceptor (7). Since the rate and extent of destruction of tocopherol during this stabilizing process vary with natural fats or fats of mixed composition (4, 8), the present experiments were undertaken to study the behavior of tocopherol in simple systems.

Materials and Methods

The materials used were *d*-*a*-tocopherol, "stable" solvents (methyl silicone,[2] polymer of trifluorovinyl chloride,[3] and dibutyl phthalate), and pure fatty acid methyl esters[4] (stearate, oleate, linoleate, and linolenate). The tocopherol was added to the solvents and/or esters in various concentrations, and these mixtures and pure tocopherol were stored at several temperatures in the dark. Periodic weighings indicated that the materials did not volatilize appreciably during the experiments. The residual tocopherol was determined at intervals by the dipyridyl method, after preliminary removal of interfering substances with sulfuric acid (6). Tests showed that this method could be used to determine tocopherol in silicone, dibutyl phthalate, and polymer of trifluorovinyl chloride as well as in fatty acid esters. The oxidation of solvents and esters was followed by determinations of peroxide oxygen (3).

Results

The stability of tocopherol in the three "stable" solvents was studied at temperatures from 60 to 120°C. (Table I). Actually silicone was the only completely stable solvent; the polymer of trifluoro-

[1] N.R.C. No. 4429 Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada.
[2] General Electric LTNV40.
[3] Fluorolube HO, from Hooker Electrochemical Company.
[4] From Hormel Institute, Austin, Minn.

TABLE I

Stability of Tocopherol in Solvents

Tocopherol concentration %	Tocopherol half-life,[a] days			
	60°C.	80°C.	100°C.	120°C.
	Methyl silicone			
0.01	170	35	7.5	3.3
0.05	165	32	5.9	1.4
0.1	139	27	5.4	1.2
0.2	130	24	4.8	1.2
	Polymer of trifluorovinyl chloride			
0.01	49	10	3.1	0.4
0.05	67	15	3.5	0.7
0.1	71	22	4.0	0.8
0.2	68	19	3.3	0.8
	Dibutyl phthalate			
0.01	54	11	3.0	0.6
0.05	75	15	3.3	1.1
0.1	91	24	4.6	1.2
0.2	46	17	4.3	1.2

[a] Although the reaction was not definable kinetically, the term "half-life" is used for convenience to indicate the time required for destruction of 50% of the original tocopherol.

vinyl chloride developed traces, and dibutyl phthalate small amounts of peroxide when stored at 120°C. The data show that the stability of tocopherol in these solvents varied directly with the resistance of the solvent to oxidation, also that the optimum level for maximum stability of tocopherol was lowest in the most stable solvent. In silicone at all temperatures the lowest concentration of tocopherol was the most stable, but in the other two solvents 0.1% was the most stable concentration studied. The destruction of tocopherol in these solvents followed an induction type of curve, with the induction phase most marked at the lower temperatures.

The stability of tocopherol in methyl esters of fatty acids was studied at 40 and 80°C. The rate of destruction of tocopherol increased with the degree of unsaturation of the ester. Apparent optimum concentrations for stability of tocopherol at 40°C. were about 0.01% or less in stearate, 0.125% in oleate, 4% in linoleate, and above 8% in linolenate (Table II). Optima at 80°C. were higher for stearate (0.25%) and oleate (2%); the other esters were not studied at this temperature. Tocopherol was less stable in a 50:50 mixture of linoleate-stearate than in oleate (of the same iodine value), and no optimum concentration was observed in the range of levels tested (Table II).

The effect of the initial presence of oxidized tocopherol on tocopherol stability in methyl oleate was next investigated. Pure tocopherol was allowed to oxidize at 80°C. until half its reducing power was destroyed. This material was then added at a concentration of 0.25% to methyl oleate, and the mixture was stored at 80°C. The "half-life" of the unoxidized portion of the tocopherol (57 days) was the same as that of an equivalent amount of pure tocopherol (0.125%) in methyl oleate under the same conditions. Thus the addition of oxidized tocopherol did not affect the stability of tocopherol in methyl oleate.

TABLE II

Stability of Tocopherol in Methyl Esters

Tocopherol concentration, %	Tocopherol half-life,[a] days at 40°C.				
	Stearate	Oleate	Linoleate	Linolenate	Linoleate-stearate, 50:50
0.01	322	20	—	—	—
0.015	290	71	—	—	—
0.075	269	168	8.0	—	—
0.125	242	191	17.7	4.9	25
0.25	206	114	27.0	5.2	60
0.5	—	—	29.3	5.5	72
1.0	—	—	32.0	5.7	82
2.0	—	—	33.0	6.0	—
4.0	—	—	33.6	7.6	—
8.0	—	—	18.5	9.8	—

[a] See note, Table I.

Addition of a stable solvent to an unsaturated ester markedly increased the stability of 1% of tocopherol dissolved in the ester. Thus in a mixture of 50% linoleate and 50% dibutyl phthalate, the half-life of the tocopherol was 39 days; in a 25:75 mixture, 57 days, and in a 12.5:87.5 mixture, 144 days at 40°C.

Tocopherol stability in the esters, and protection given to the esters by the tocopherol, appeared to be directly related. The order of lengthening of the induction period of the esters (time to most rapid stage of peroxide development) by the various concentrations of tocopherol was the same as the order of stability of the tocopherol concentrations themselves (examples in Figures 1 and 2 for oleate and linoleate at 40°C.). Although the rate of oxidation of the esters increased with increasing unsaturation, peroxide development in stearate was similar to that in oleate and results for linolenate were similar to those for linoleate.

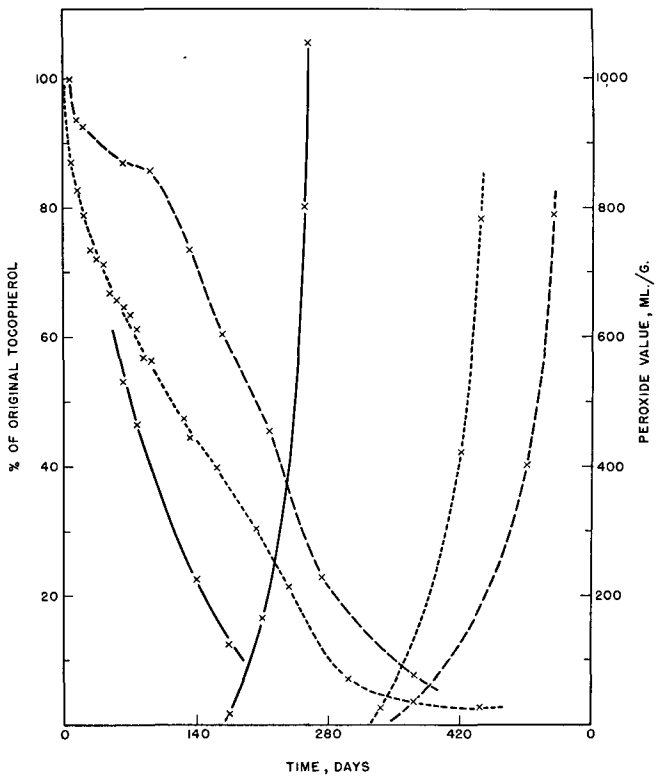

FIG. 1. Stability of tocopherol in methyl oleate. Descending curves (left), tocopherol retention (% of original amount added); ascending curves (right), peroxide accumulation (ml. of 0.002 *N* thiosulfate per g.). x——x, 0.015% tocopherol; x— — x, 0.125% tocopherol; x- - - - - x, 0.25% tocopherol.

The amount of tocopherol remaining at the end of the induction period varied with the ester. In oleate (Figure 1) and in stearate (not shown) less than 15% of each original tocopherol concentration remained at the end of the induction period. In linoleate (Figure 2) and linolenate (not shown) up to 50% of the original tocopherol was still present, and the percentage remaining varied with the original concentration.

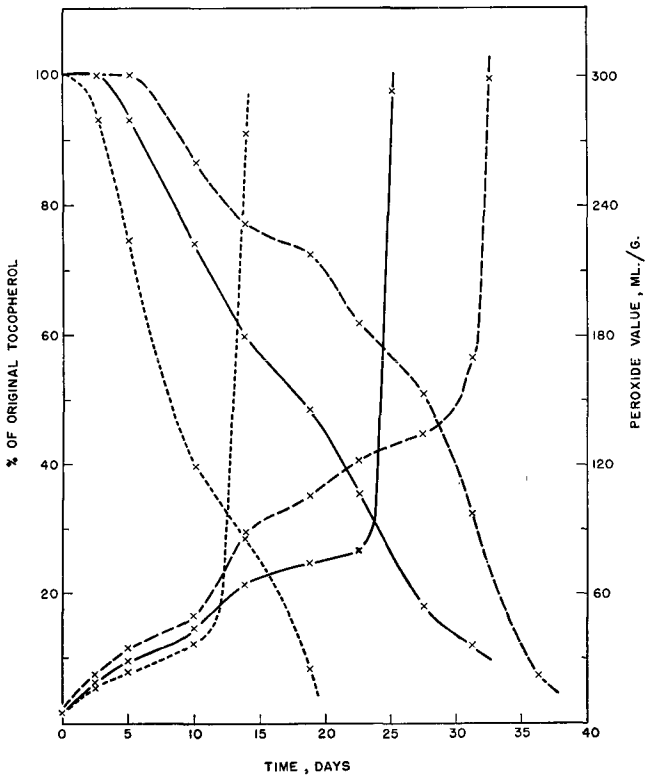

FIG. 2. Stability of tocopherol in methyl linoleate. Descending curves (left), tocopherol retention (% of original amount added); ascending curves (right), peroxide accumulation (ml. of 0.002 *N* thiosulfate per g.). x - - - - - x, 0.075% tocopherol; x —— x, 0.125% tocopherol; x — — x, 0.25% tocopherol.

Storage of pure tocopherol showed that its stability was approximately halved with each 10°C. rise in temperature (Table III) and that, as with tocopherol in stable solvents, the destruction followed an induction type of curve. Pure tocopherol was less stable

TABLE III

Stability of Pure Tocopherol

Temperature, °C.	Half-life,[a] days
40	113
60	29
80	8.2
100	2.8
120	0.9

[a] See note, Table I.

than tocopherol in stable solvents (*cf.* Tables I and III). A preliminary examination of the oxidized tocopherol by infrared absorption and by micromolecular distillation indicated that a variety of products was formed, but formation of a γ-lactone and polymers was observed at all storage temperatures. A dimer (5) and a γ-lactone (2) have previously been found in tocopherol oxidized by chemical means.

No simple kinetic description for the rate of destruction of tocopherol in any of these experiments could be obtained.

Discussion

Optimum concentration effects of tocopherol in the stabilization of fats are well known (1, 10). Early work with lard (4) indicated that tocopherol was completely destroyed during the induction period and that the destruction of tocopherol was a first-order reaction. Subsequent studies on vegetable oils (9) showed that appreciable amounts of tocopherol could remain at the end of the induction period and that the disappearance of tocopherol was not a simple process.

The present results suggest that some of the earlier, observed differences can be explained in terms of fatty acid composition. Elimination of tocopherol during the induction period of lard oxidation appears to be related to the low content of acids more unsaturated than oleic. In vegetable fats the relatively high content of linoleic and/or linolenic acids would favor a higher tocopherol retention at the end of the induction period. The present results support the view (8) that the loss of phenolic antioxidants does not occur as a single uncomplicated reaction.

The optimum level for tocopherol stability and the rate of tocopherol destruction depend on the nature of the medium as well as on total unsaturation. Tocopherol was less stable and less effective in the more unsaturated esters, but dilution with a stable solvent increased the stability of tocopherol. Pure tocopherol was less stable than tocopherol in a stable solvent. Therefore, since tocopherol itself is readily oxidizable, it should be considered as forming part of the unstable material in the medium.

Summary

The stability of *d*-α-tocopherol in stable solvents and in methyl esters of fatty acids varied with the stability of the medium and with the concentration of tocopherol. Pure tocopherol was less stable than tocopherol in solvents. The concentrations of tocopherol that were most effective in delaying rapid oxidation of the esters were also best for stability of tocopherol itself. The rate of destruction of tocopherol could not be described by simple kinetics.

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