vegetable oils are usually alkali-refined, traces of soap alkali, or sodium salts of phospholipids may frequently be present. These compounds may interfere with the normal antioxygenic activity of tocopherols and phospholipids but, conversely, may react in the presence of some acidic types of compounds, such as tartaric, citric, ascorbic, or phosphoric acid, to permit the normal antioxygenic action of the tocopherols and phospholipids. Furthermore, with some acidic compounds, the antioxygenic effect may even be enhanced.

These results have demonstrated that fatty acid monoesters of l-ascorbic and d-isoascorbic acids are capable of exerting a synergistic action with other antioxidants in delaying the onset of rancidity in fats as tested under certain accelerated conditions. It is realized that the requirements to be met by a commercial antioxidant for use in edible fats and oils are manifold. Thus far, time has not permitted us to evaluate the esters beyond the points discussed in this paper, but in the near future experiments will be conducted to determine the practical value of these combinations.

Conclusions

The results of this investigation emphasize the possibility of greatly improving the keeping quality of lard by rendering and refining under conditions that do not destroy natural antioxidants such as phospholipids, tocopherols, and possibly other unknown naturally occurring inhibitors. Furthermore, the importance of removing all traces of soap and alkali from refined fats and oils is again emphasized. On the other hand, it seems doubtful whether even the best commercial handling of the animal fats will give a product having the high degree of stability frequently attained in commercial hydrogenated vegetable shortenings. However, by the addition of suitable, carefully selected, edible antioxidants capable of exerting a synergistic effect, it is possible to increase the stability of lard and edible tallow to such an extent that it compares favorably with the best of the fats available.

Summary

1. The fatty acid monoesters of l-ascorbic and d-isoascorbic acids have been tested for antioxygenic activity in different fats and oils under various conditions and in combination with other inhibitors.

2. Traces of soap were found to have a deleterious effect on the stability of fats. This deleterious effect can be counteracted by the use of fat-soluble ascorbyl monoesters.

3. The ascorbyl monoesters used in combination with either α-tocopherol or phospholipids, or both, show a marked synergistic antioxidant effect.

4. Possible explanations are given for certain synergistic phenomena that have been observed.

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The Fats and Phosphatides In Grass

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One of the most important animal foods is ordinary grass, and it is rather remarkable that so little is known about the composition of a food of such first class importance.

There seems to be only a few publications on research work, and those by Chibnall and co-operators (1) cover only a small proportion of the fatty matter present in grass. They only extracted the dry material with ether, and ether is not a good solvent if large quantities of phosphatides are present. The use of ether as the only solvent has two other disadvantages, it dissolves only a certain proportion of the phosphatides, besides the fat, and also all the coloring matter, i.e. chlorophyll as well as carotin. It is rather difficult to purify this mixture of coloring and fatty matter. The quantities of phosphatides extracted by this method are rather small and it seems that they still contain some foreign matter. They obtained only 56 grams of crude phosphatides = 0.25%, from 22 kg. dry grass, but about half of it (45% to be exact) was water soluble, after treatment with HCl, and only about 0.12% phosphatides were estimated which has a rather high amount of Ca and Mg and a low amount of N. and P.; the N content, especially, was very small, only 0.63%.

It is a well-known fact, and has been proved by many experiments, that usually only a small amount —about 30-40%—of the total phosphatide present in the animal or plant raw material can be extracted by the ordinary fat solvents—ether, petrolether, etc. This is due to the "physical" linkage between phosphatides and proteins or sugars, and only by using alcohol can this "bond" be dissolved. Therefore it was to be expected that by using ether as a solvent only a certain quantity of the phosphatides could be found in the extract, only the so-called "free" phosphatide, and that another portion was left behind.

It was therefore decided to use the following method, which has the advantage that most of the fatty and coloring matter should be eliminated before the principal quantities of phosphatides were dissolved.

The extraction was carried out in three stages: 1. Acetone; 2. Petrolether; 3. Alcohol and benzol (20:80).

Acetone was chosen because it is a very good solvent for fat, chlorophyll, carotin, and other coloring products, but a very bad solvent for phosphatides, especially if the extraction is made in the cold. It is well known that acetone, in the presence of other products—fats, etc., always dissolves a small quantity of phosphatides and it was therefore to be expected that there must be some P. in the acetone extract.

The second solvent, petrolether, was chosen to extract most of the "free" phosphatides not extracted with acetone. It was hoped that this petrolether would be less colored than the very dark green acetone extract and that it would be easier to get purer products.

The third solvent, the mixture of alcohol and benzol, should give another quantity of phosphatides, besides wax and a certain quantity of fat, which always escapes the extraction with ether and petrolether. It has been proved that by this method, quite considerable quantities of phosphatides can be extracted and that the total amount of "fatty" matter of all kinds, including wax, is rather high, higher than was to be expected.

The raw material used was an ordinary, finely powdered, grass meal, of a dark green color. This mael was dried in a special grass-drying plant in such a way that in a very short time all the moisture was removed without influencing any of the vitamins, especially carotin. Five hundred grams of this grass were extracted in the above mentioned manner and the results were as follows:

1. Acetone Extract	= 23.0 gr.	= 4.6%
2. Petrolether Extract	= 2.05 gr.	= 0.4%
3. Alcohol and Benzol Extract (after dissolving in petrolether)	= 11.7 gr.	= 2.3%
		7.3%

I do not think that it was known that ordinary grass has such a high percentage of fat, phosphatides, wax, chlorophyll and other products soluble in the ordinary solvents.

Each of these extracts were examined to find out, as far as possible, its composition—its content of phosphatides, fat, etc.

1. *Acetone Extract.* This was dissolved in ether to remove traces of a brownish crystalline substance, found to be carbohydrates. The clear etherial solution, after distilling off the solvent, gave a fat-like dark green product, nearly solid in the cold but liquid at water-bath temperature. The estimation of this product gave the following figures:

Total ash	1.5%
Silica	nil
Iron	nil
Copper	nil
Ca.	0.105%
Mg.	0.18%
P.	0.5%

It contains, therefore, only a very small percentage of "phosphatides," even if it was assumed that all the P. was due to phosphatides—ordinarily, phosphatides contain between 3.7-4.0% P., so it was not regarded worth while to separate the phosphatides, if any, from the acetone extract. It was therefore proved that acetone mostly dissolves the other products and eliminates most of the by-products, which makes the purification of the phosphatides rather difficult.

2. *Petrolether Extract.* The petrolether extract, small in quantity, had a very high percentage of P. It was only very slightly green in color. The ether solution was precipitated with acetone; the acetone insoluble part contained 3.00 P. = approximately 80% phosphatide; the acetone soluble part = 1.11% P. = approximately 28% phosphatide. It was therefore quite obvious that most of the "free" phosphatides were extracted in this way, and it was even possible, although the total quantity was very small, to separate a highly purified phosphatide.

3. *Alcohol Benzol Extract.* This was the most interesting extract. It was dissolved again in petrolether to remove the sugars; most of the wax also remains. The sugars, which are soluble in water, can be separated from the wax by treatment with water.

a.) The clear petrolether extract is concentrated and poured into acetone; immediately a nearly white precipitate is flocculated out. After 24 hours in the cold the precipitate has settled, the clear acetone liquor can then be decanted and filtered. The precipitate is washed repeatedly with acetone. The residue is a dark brown paste with no green color at all. It is easily soluble in ether. The analysis gave the following figures:

Total ash	6.88%
SiO_2	nil
P.	2.80%
N.	1.72%
Ca.	0.25%
Mg.	0.18%

Total quantity = 2.03 gr. = 0.406%
Ratio — N : P = 1 : 1.6

This petrolether extract seems to be a nearly pure phosphatide; it still contains, as usual, small quantities of sugar, which are always rather difficult to remove, when dealing with plant material. The presence of small quantities of Ca. and Mg. may be due, to some extent, to products first mentioned by Chibnall, consisting of Ca. and Mg. salts of a "phosphatide" where the base has been replaced by these metals, but this is rather unlikely because the amount of N. is rather high. It may be that small quantities of chlorophyll are still present. According to Willstatter (2), chlorophyll contains 2.70% Mg., therefore, it is possible that about 5% chlorophyll remains in this product; it is extremely difficult to remove the last traces of colored matter. Anyway, it is quite obvious that rather large quantities of phosphatides can only be extracted with a solvent which can split the "bindings" of phosphatides with other products, i.e. sugars or proteins.

b.) The acetone soluble part — the oil — is rather dark in color, it is almost solid, the iodine value—47.2—is extremely low, so that it must contain a large quantity of saturated fatty acids.

c.) The petrolether insoluble part is a wax, easily soluble in trichlorethylene; it is 6.245 gr. = 1.25% of the raw material.

Conclusion

The analysis of grass, in a dry state, proved that it contains a rather high amount of fat and fat-like products, phosphatides and waxes; the total quantity is over 7%. Most of the phosphatides can only be extracted with a solvent mixture containing alcohol. There is no doubt that really true phosphatides can be separated, but it seems that other P-containing organic compounds are present in small quantities. The total amount of phosphatides is at least 0.5% in the dry grass.

I desire to express my thanks to Mr. Charles M. Caines who has been of great assistance in conducting

a good deal of the experimental analytical work connected with this research.

Grass Meal

Total quantity of fatty matters, extracted with solvents from 500 grams dried meal:

Acetone extract	= 23.0 gr.	= 4.6%
Petrolether extract	= 2.05 gr.	= 0.41%
Alcohol and Benzol extract	= 11.7 gr.	= 2.34%
	36.75 gr.	= 7.35%

Acetone Extract

Total ash	1.5%
Silica	nil
Iron	nil
Copper	nil
Ca.	0.105%
Mg.	0.180%
P.	0.50%

Petrolether Extract

a.) Acetone insoluble part (Phosphatide) contains 3.00% P. (80% Phosphatide).

b.) Acetone soluble part (fat) contains 1.11% P. (28% Phosphatide).

Alcohol and Benzol Extract

Petrolether Extract:

P.	2.80%
N.	1.72%
SiO_2	nil
Ca.	0.25%
Mg.	0.18%
Total ash	6.88%

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Formation and Decomposition of Peroxides of Unsaturated Fat Esters

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Introduction

The reaction of molecular oxygen with unsaturated fats, fat acids, or esters to form peroxides has in recent years become the most generally accepted mechanism for the primary reaction of oxygen upon the unsaturation present in these compounds. This autoxidation is a fundamental factor in reactions such as rancidification, film formation in paints, and the formation of blown oils for technical uses. The present study was undertaken as a preliminary investigation to determine the effect of temperature on the formation and decomposition of these peroxides. It was felt that a knowledge of optimum conditions for formation and of the rate of decomposition at various temperatures was necessary before detailed studies of their decomposition products, or their possible isolation and characterization, could be undertaken. Distilled methyl esters of soybean fat acids were used because they were easy to prepare and could be readily purified by distillation. A similar study using the esters of pure individual unsaturated fat acids would obviously be highly desirable. However, the difficulty of preparing the pure esters in sufficient quantities for the exploratory work led us to use the mixed methyl esters prepared from soybean oil, with the expectation that data on such a mixture would be of considerable value in planning future work on pure compounds. Since the study on pure compounds has been postponed, due to the urgency of other work, the data on the mixed esters are presented at this time.

Experiments and Discussion

Hydroxyl numbers were determined by the acetic anhydride-pyridine method of West, Hoagland, and Curtis (1), with 24-hour reaction time at room temperature. Butanol was not added and aqueous alkali was used, with vigorous shaking of the resulting emulsion at the end-point. The phenolphthalein end-point was easier to observe in the emulsion than in the red-brown colored homogeneous solution ordinarily obtained. Highly peroxidized esters always developed this color with the pyridine reagent.

Acid numbers were calculated from the blank titration (pyridine with no anhydride) of the hydroxyl number determination.

Iodine number. Four different methods for determination of iodine number were tested on a sample of fresh esters and on one which had been blown with oxygen for 24 hours at 55° C. The results are shown in Table 1.

The Kaufmann method (200 percent excess and 4-hour reaction) agreed well with the Wijs method and was subsequently used because of convenience in preparation of the reagent and in handling a large number of samples at one time. It may be of interest to note that with oxidation there was a two- or threefold increase in the difference between the Hanus and Kaufmann iodine numbers.

Peroxidized esters showed a "repeating" end-point to a degree proportional to the peroxide content. Titrations were made immediately after the addition of the potassium iodide solution and water to the reagent to minimize the liberation of iodine by the peroxides before completion of the titration. On an oxidized sample, titration of the excess bromine by arsenious acid solution (5) gave a value for iodine number (93.7) very close to that obtained by the usual iodide-thiosulfate method (93.9). This would indicate that the liberation of iodine from iodide in the latter method was negligible, or that the peroxides oxidized

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