# Fatty Acid Monoesters of l-Ascorbic and d-Isoascorbic Acids as Antioxidants for Fats and Oils

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The prevention of rancidity in fats and foodstuffs has been recognized for years as a problem of great importance from the standpoint of both health and economy. It assumes even greater significance now with our nation at war. From the standpoint of health, rancidity is serious because oxidized or rancid fat not only impairs the palatability of the food in which it is incorporated but it also destroys certain essential vitamins. Economically, the spoilage of fats, resulting in their diversion from edible to inedible channels, causes serious losses.

That lard has been decreasing in favor for commercial and household use may be inferred from the fact that up until 1937 the price of lard was usually higher than that of prime summer yellow cottonseed oil but since 1937 this has not been true. One reason for this unfavorable trend is that lard inherently is more susceptible to oxidative rancidity than are vegetable shortenings. This instability is characteristic of most animal fats. To place lard and other animal fats on a better competitive basis, serious attention is being given to the problem of preventing rancidity.

Attempts to solve the problem of retarding oxidative rancidity by the addition of natural or synthetic inhibitors have been fairly successful. Of considerable interest is the recent work of Olcott (1) and Golumbic (2), in which an acidic type of inhibitor, e.g., ascorbic acid, and a phenolic type, such as $\alpha$-tocopherol, used together give an increased protection greater than the additive effect of the two substances used singly. The use of ascorbic acid and its isomers as antioxidants for aqueous emulsions of fats has been patented (3). Since ascorbic and isomeric ascorbic acids are practically insoluble in substantially dry fats and oils technical difficulty arises in their use. It occurred to us that if fat-soluble derivatives of these compounds could be prepared without affecting the enediol group, presumably the functional antioxygenic group, their use as oxidation inhibitors might be of more general application. Accordingly, fat-soluble derivatives, such as the lauric, myristic, palmitic, and stearic acid monoesters of l-ascorbic and d-isoascorbic acids were prepared. A complete report on the preparation of these esters is being published separately.

These ascorbyl esters have been investigated for antioxygenic activity, singly, and in combinations with other compounds, such as sodium bicarbonate, sodium stearate, $\alpha$-tocopherol, and soybean phospholipids (commercial soybean lecithin). The results of this work form the basis of the present report.

## Experimental

An oven test was used to obtain the data given in Tables I and II. This test consisted of heating 20-cc. samples of fat or oil, contained in 50-cc. low-form beakers covered with watch glasses, in a thermostatically controlled, gravity-convection oven at 100° C. At intervals organoleptic tests and peroxide determinations were carried out. The stability was estimated as the time required for the sample to attain a peroxide value that was indicative of incipient rancidity. These peroxide values, expressed in milli-mols of peroxide oxygen per kg. of sample, were as follows: For lard, 10; for hydrogenated vegetable shortening, 15; for peanut oil, 35; and for cottonseed oil, 40.

The stability values given in Tables III, IV, V, and VI were obtained by the Active Oxygen Method at 100° C., sometimes called the Swift stability test. This method was described by King, Roschen, and Irwin (4). Several changes, however, were introduced in the method and apparatus. Instead of using three 20-cc. portions of each sample placed in the bath one hour apart, only one 20-cc. portion was used. At the first indication of any "off" odor, approximately 0.5-gm. portions were removed for peroxide determinations. The tests were continued in this manner until the samples were definitely rancid. During this time at least three samples were taken for peroxide determinations. The stability, to the nearest half hour, was then estimated in the usual manner from the peroxide-time curves. These stability values were found to be reproducible and to agree with those obtained by the three-tube method. Aeration tubes with ground glass joints were substituted for rubber-stoppered test tubes. Provision was also made to prevent clogging of the capillaries by entrained moisture, which at times condensed from the washed air stream. Details of these changes will be published separately.

The monoesters of l-ascorbic and d-isoascorbic acids used in these investigations were pure compounds unless otherwise indicated. For convenience, the term "ascorbyl esters" is used to indicate the monoesters of either l-ascorbic or d-isoascorbic acids. All lards used in this investigation were fresh commercially prepared samples, except the kettle-rendered lard (Table VI), which was purchased on the local retail market.

TABLE I

Effect of Equivalent Amounts of Different Ascorbyl Monoesters on the Stability of Drip-Rendered Lard (A)—Oven Test at 100°C.

| Inhibitor | Concentration of Inhibitor | Stability |
|---|---|---|
| | *Percent* | *Hours* |
| Control | | 7 |
| l-ascorbyl laurate | 0.10 | 15 |
| l-ascorbyl myristate | 0.11 | 17 |
| l-ascorbyl palmitate | 0.12 | 15 |
| l-ascorbyl stearate | 0.13 | 15 |
| d-isoascorbyl laurate | 0.10 | 16 |
| d-isoascorbyl myristate | 0.11 | 14 |
| d-isoascorbyl palmitate | 0.12 | 15 |
| d-isoascorbyl stearate | 0.13 | 15 |

From the data in Table I, it is apparent that the various ascorbyl esters had about the same activity in this sample of lard. In subsequent tables the results obtained with d-isoascorbyl palmitate or stearate will be used as representative of the whole series of esters, although in many cases several esters were tested.

The ascorbyl esters were added to different fats and oils to determine their general applicability as antioxidants. The results are given in Table II.

TABLE II

Effect of d-Isoascorbyl Palmitate on the Stability of Different Fats and Oils—Oven Test at 100°C.

| Substrate | Concentration of Inhibitor | Stability | Increase in Stability |
|---|---|---|---|
| | *Percent* | *Hours* | *Hours* |
| Lard (A), Drip-rendered | Control | 7 | .... |
| Lard (A), Drip-rendered | 0.05 | 14 | 7 |
| Lard (A), Drip-rendered | 0.24 | 28 | 21 |
| Peanut Oil, Alkali-refined | Control | 34 | .... |
| Peanut Oil, Alkali-refined | 0.05 | 56 | 22 |
| Peanut Oil, Alkali-refined | 0.24 | 76 | 42 |
| Cottonseed Oil, Alkali-refined | Control | 18 | .... |
| Cottonseed Oil, Alkali-refined | 0.05 | 42 | 24 |
| Cottonseed Oil, Alkali-refined | 0.24 | 76 | 58 |
| Vegetable Shortening (Hydrogenated) | Control | 21 | .... |
| Vegetable Shortening (Hydrogenated) | 0.05 | 50 | 29 |
| Vegetable Shortening (Hydrogenated) | 0.24 | 77 | 56 |

In general, these data show that the esters are somewhat more effective in vegetable oils than in the sample of lard used, probably owing to a synergistic action with natural inhibitors, as will be shown later.

Similar tests made on different samples of lard, however, gave rather unexpected results, as indicated by the data in Table III.

TABLE III

Effect of d-Isoascorbyl Monoesters on the Stability of Different Lards—Active Oxygen Method

| Substrate | Concentration of Inhibitor | Stability |
|---|---|---|
| | *Percent* | *Hours* |
| Lard (A), Drip-rendered | Control | 2 |
| Lard (A), Drip-rendered | 0.12 | 3 |
| Lard (A), Drip-rendered | 0.47 | 22 |
| Lard (B), Drip-rendered | Control | 2 |
| Lard (B), Drip-rendered | 0.12 | 15 |
| Lard (B), Drip-rendered | 0.47 | 136 |
| Lard (C), Prime Steam | Control | 2 |
| Lard (C), Prime Steam | 0.47 | 2 |
| Lard (D), Prime Steam | Control | 3 |
| Lard (D), Prime Steam | 0.47 | 2½ |
| Lard (E), Prime Steam | Control | 2½ |
| Lard (E), Prime Steam | 0.47 | 2 |
| Lard (F), Prime Steam | Control | 4 |
| Lard (F), Prime Steam | 0.12 | 6½ |

The behavior of the ascorbyl esters with prime steam lard is in striking contrast to that observed with drip-rendered lard. Attempts to determine the reason for this led to an interesting series of observations. Several clues were available. First, it was known that the drip-rendered lard had been refined by treatment with sodium bicarbonate and active carbon, whereas the prime steam lard had not. Second, the aeration during the stability testing of the drip-rendered lard produced excessive foaming, which was thought to be due to either phospholipids, traces of soap from the bicarbonate used in refining, or possibly a combination of the two. It was also considered possible that the strong antioxygenic action of the ascorbyl esters in drip-rendered lard might have been due to a mutual retarding action with a trace of tocopherol since that inhibitor has been reported by Karrer, et al. (5) to be a normal constituent of swine fat. In other words, it was reasoned that one difference between the prime steam lards and drip-rendered lards might be that in the former most of the tocopherol and phospholipids had been lost or destroyed in the steam-rendering process, whereas in the drip-rendered lard, small but none the less significant amounts of the natural antioxidants had been retained. Another difference might be due to the soap or bicarbonate in the drip-rendered lard resulting from the refining treatment.

To test this explanation, attempts were made to simulate drip-rendered lard by the addition to prime steam lard of small amounts of sodium bicarbonate, sodium stearate, phospholipids, and α-tocopherol in different combinations, and to determine the effect of ascorbyl esters added to these mixtures. The results are given in Tables IV and V.

TABLE IV

Effect of Small Amounts of Soap and Sodium Bicarbonate on the Stability of Prime Steam Lards—Active Oxygen Method

| Test No. | Substrate and Substance Added | Stability |
|---|---|---|
| | | *Hours* |
| 1 | Prime Steam Lard (C) (Control) | 2 |
| | + 0.1% sodium bicarbonate | 1 |
| 2 | Prime Steam Lard (D) (Control) | 3 |
| | + 0.08% sodium stearate + 0.02% sodium bicarbonate | 2½ |
| 3 | Prime Steam Lard (E) (Control) | 2½ |
| 3-a | + 0.03% sodium stearate + 0.005% sodium bicarbonate | 1 |
| 3-b | + 0.03% phospholipids | 5½ |
| 3-c | + 0.03% phospholipids + 0.03% sodium stearate + 0.005% sodium bicarbonate | 2 |
| 3-d | + 0.001% α-tocopherol | 4 |
| 3-e | + 0.001% α-tocopherol + 0.03% sodium stearate + 0.005% sodium bicarbonate | 1½ |
| 3-f | + 0.01% sodium stearate + 0.001% sodium bicarbonate | 2 |

In every instance, as shown in Table IV, the soap and sodium bicarbonate reduced the stability of the lard, even in tests where phospholipids or α-tocopherol had also been added. In further tests, not given in the table, 0.47 per cent of ascorbyl ester was added to each of another series of mixtures of the same compositions. A positive antioxygenic effect was obtained in each instance, and in some, remarkable stability was produced. Thus, the addition of the ascorbyl ester to the materials used in tests 3-a, 3-b, 3-c, and 3-e of Table IV increased the stability to more than 56 hours. These observations taken as a whole are interpreted to mean that probably nearly all the natural phospholipids and tocopherols had been destroyed in the steam-rendering process used in making these prime steam lards, whereas the drip-rendered lards (Table III) still contained at least some phospholipids, which produced a synergistic antioxidant effect with the ascorbyl esters added to these lards. These drip-rendered lards also contained small amounts of sodium salts, possibly soap or traces of bicarbonate, or the sodium salt of the phospholipids, which in some way interfered with the normal antioxygenic activity of the phospholipids present. The ascorbyl esters not only counteracted this deleterious action of the sodium salts but also showed enhanced antioxidant activity with sodium stearate. The phosphorus content of the drip-rendered lard was high in comparison with that of the prime steam lard (D), 0.0013 per cent as against 0.00006 per cent; nitrogen was present in the former but was absent in the latter. This is further evidence of the presence of phospholipids in the drip-rendered lard.

The synergistic antioxidant action of the ascorbyl esters with phospholipids and with α-tocopherol in-

dicated by these results was further investigated with lower concentrations of the esters. These results are shown in Table V.

TABLE V

Effect of d-Isoascorbyl Monoester,* α-Tocopherol, and Soybean Phospholipids on the Stability of Prime Steam Lard—Active Oxygen Method

| Substance Added | Stability |
|---|---|
| | *Hours* |
| Prime Steam Lard (F) (Control) | 4 |
| + 0.06% d-isoascorbyl monoester | 4 |
| + 0.12% d-isoascorbyl monoester | 6½ |
| + 0.03% phospholipids | 7½ |
| + 0.03% phospholipids + 0.06% d-isoascorbyl monoester | 26 |
| + 0.03% phospholipids + 0.12% d-isoascorbyl monoester | 44 |
| + 0.001% α-tocopherol | 4½ |
| + 0.001% α-tocopherol + 0.06% d-isoascorbyl monoester | 7 |
| + 0.001% α-tocopherol + 0.03% phospholipids | 10 |
| + 0.001% α-tocopherol + 0.03% phospholipids + 0.06% d-isoascorbyl monoester | 40 |
| + 0.001% α-tocopherol + 0.03% phospholipids + 0.12% d-isoascorbyl monoester | 60 |

* Commercial stearic acid containing appreciable amounts of palmitic acid was used in the preparation of this monoester.

It is clear that a number of synergistic effects were produced.

1. d-isoascorbyl monoester with α-tocopherol
2. α-tocopherol with phospholipids
3. d-isoascorbyl monoester with phospholipids
4. d-isoascorbyl monoester with α-tocopherol + phospholipids

The first type of synergism might be expected from the work of Olcott (1) and Golumbic (2) and the second type might be expected from the work of Swift, et al. (6) and Olcott and Mattill (7). However, the synergistic effects of the last two combinations merit attention, not only because of the magnitude of the effect produced by use of the ester with another component but also because of the "compound effect" when it is used with two additional components.

In order to make certain that this action was not due to a peculiarity in one particular sample, similar tests were carried out in which the ascorbyl monoesters and soybean phospholipids were used on a series of lards and a sample of oleo oil. Typical results are shown in Table VI.

TABLE VI

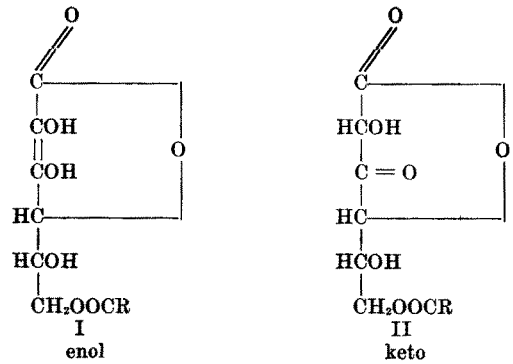
Effect of d-Isoascorbyl Monoester and Soybean Phospholipids on the Stability of Various Cooking Fats—Active Oxygen Method

| Substrate and Substance Added | Stability | Increase in Stability |
|---|---|---|
| | *Hours* | *Hours* |
| Prime Steam Lard (G) (Control) | 7½ | .... |
| + 0.06% d-isoascorbyl monoester | 5 | —2½ |
| + 0.03% phospholipids | 10 | 2½ |
| + 0.06% d-isoascorbyl monoester + 0.03% phospholipids | 31 | 23½ |
| Prime Steam Lard (H) (Control) | 9½ | .... |
| + 0.06% d-isoascorbyl monoester | 14 | 4½ |
| + 0.03% phospholipids | 12 | 2½ |
| + 0.06% d-isoascorbyl monoester + 0.03% phospholipids | 50 | 40½ |
| Prime Steam Lard (I) (Control) | 7 | .... |
| + 0.06% d-isoascorbyl monoester | 13 | 6 |
| + 0.03% phospholipids | 11 | 4 |
| + 0.06% d-isoascorbyl monoester + 0.03% phospholipids | 52 | 45 |
| Kettle-rendered Lard (Control) | 1 | .... |
| + 0.06% d-isoascorbyl monoester | 3 | 2 |
| + 0.03% phospholipids | 1 | 0 |
| + 0.06% d-isoascorbyl monoester + 0.03% phospholipids | 3 | 2 |
| Edible Oleo Oil (Control) | 7 | .... |
| + 0.06% d-isoascorbyl monoester | 6 | —1 |
| + 0.03% phospholipids | 19 | 12 |
| + 0.06% d-isoascorbyl monoester + 0.03% phospholipids | 69 | 62 |

The data in Tables V and VI show that the stability of high quality prime steam lard can be increased to a level comparable with that of the usual hydrogenated vegetable shortenings by the addition of very small amounts of phospholipids and ascorbyl monoesters. The addition of minute amounts of α-tocopherol further increases the stability. The lard of low stability, however, was not greatly improved.

## Discussion

The antioxidant activity of the isomeric ascorbic acids and their monoesters is thought to be due to the enediol group —C(OH)=C(OH)—. The formulas for d-isoascorbyl monoester may be written as follows, assuming esterification took place on the primary alcohol group:




Several alternative explanations exist for the enhanced antioxidant activity of these esters in the presence of certain other substances.

Olcott and Mattill (8) have advanced an explanation for the synergistic action of ascorbic acid when used with tocopherol. They have found that ascorbic acid is not appreciably oxidized by fat peroxides and does not prevent their formation, but it retards the oxidation of tocopherol, which in turn temporarily prevents the oxidation of the fat. A similar explanation might be applied to those cases of synergism which we have observed when using the esters in combination with tocopherol and the phospholipids.

This explanation does not suffice, however, in the case we have observed when a synergistic action occurs between soap and the ester when both are added to a fat. The presence of traces of alkali may favor an enolic form, similar to formula I, which might be expected to have greater reducing properties and greater antioxidant properties than formula II. An alternative explanation might be based on the suggestion of Bailey and French (9) that surface activity may play an important part in the effectiveness of an inhibitor. It is generally conceded that autoxidation of fats takes place at the liquid or fat-oxygen interface. Then it seems reasonable to suppose that a trace of inhibitor could exert its greatest effect when present at the interfacial surfaces and that only a relatively small concentration of inhibitor at these surfaces would exert a great effect. However, owing to lack of fundamental information regarding surface-active phenomena in fat or oil media, it is difficult to come to any definite conclusions concerning this suggestion.

Some of the observations of Olcott may be explained in the light of the results given in this paper. Since

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