

STUDIES ON RANCIDITY OF BUTTERFAT. PART II. THE USE OF L-ASCORBYL ESTERS OF FATTY ACIDS AS ANTIOXIDANTS

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A method for the preparation of *l*-ascorbyl esters of fatty acids in good yield is described. The antioxidant activity of these esters have been tested with butterfat both by storage experiments and by Swift's stability test. The biological availability of these esters has also been tested by carrying out assay with guinea-pigs. The loss of *l*-ascorbyl esters within the induction period has been determined.

In a previous paper (Mukherjee and Goswami, this *Journal*, 1947, 24, 239) of this series it has been shown that *l*-ascorbic acid possesses good antioxidant properties, particularly when used in combination with other antioxidants, but its use in the case of fat is limited because of its insolubility in the same. The fact that esters of *l*-ascorbic acid with fatty acids like stearic, palmitic etc. are quite soluble in fat, makes its incorporation much easier in such forms. Hence, attempts were made to prepare the respective *l*-ascorbyl esters of the following fatty acids: stearic, palmitic, myristic and lauric. The method originally employed by Swearn, Sterton, Turner and Wells (*Oil & Soap*, 1943, 20, 224) was found to give very low yields and in most cases the esters could not be prepared using a 16-hour reaction time employed by them. A detailed study regarding proportions of the reactants, time of reaction, effect of mechanical agitation and temperature has finally led to a successful preparation of these esters in quantity. The method employed is outlined briefly as follows.

EXPERIMENTAL

About 25 g. of the finely powdered fatty acid was added to 250 c.c. of concentrated H_2SO_4 containing 30.0 g. of *l*-ascorbic acid in solution at 30° with thorough stirring till the solution became clear; it was necessary to cool the mixture externally during the reaction. The reaction mixture was then shaken for 96 hours at room temperatures (34° - 37°) in a mechanical shaker. During this period the whole solution became dark coloured. The solution was then slowly added to about 500 g. of crushed ice with thorough stirring till the oily liquid solidified. The mixture was then allowed to attain room temperature and extracted with ether, ethereal layer washed free from sulphuric acid, dried over anhydrous Na_2SO_4 , and ether distilled off; the residual solid obtained was purified by repeated recrystallisation according to Swearn *et al.* (*loc. cit.*) except that the treatment with charcoal was found necessary to obtain a perfectly white crystalline product. The melting points of the esters, when dried in a vacuum desiccator over P_2O_5 , however, were found to be identical with those reported by Swearn *et al.* (*loc. cit.*). The yield of the purified ester varied from 50-60%.

The effect of these four esters on the retardation of rancidity of butterfat was determined both by the Swift fat stability test (King, Roschen and Irwin, *Oil & Soap*, 1933, 10, 105) and by storage experiments. Table I records the results obtained with the first method, the end of the induction period being taken as the time necessary to reach a peroxide value of 10.0

TABLE I

Antioxidant activity of fatty acid esters of l-ascorbic acid on butterfat (Swift stability test) at 100°.

	Antioxidant.	Conc.	Induction period.	Protection factor.
1	<i>l</i> -Ascorbyl stearate	0.01%	45 hrs.	1.25
2	„ palmitate	0.01	48	1.40
3	„ myristate	0.01	50	1.50
4	„ laurate	0.01	52	1.60
5	Control	—	20	—

It appears from Table I that the esters lengthen the induction period to some extent. But it should be borne in mind that these fatty acid esters of *l*-ascorbic acid are very much liable to oxidation, particularly under the drastic conditions which are employed in the Swift stability test. So, from the positive values of the protection factor obtained above, it can safely be expected that the degree of protection will be much higher with actual storage experiments the results of which are detailed below.

TABLE II

Antioxidant activity of fatty acid esters of l-ascorbic acid on butterfat on storage at 37°.

Antioxidant.	Conc.	Peroxide value after days at 37°.						
		0.	15	30.	45.	60	90.	120.
1. <i>l</i> -Ascorbyl stearate	0.01%	0.0	0.0	0.02	0.10	0.2	1.16	5.74
2. „ palmitate	0.01	„	„	0.02	0.10	0.2	1.0	4.83
3. „ myristate	0.01	„	„	0.03	0.13	0.2	1.2	5.9
4. „ laurate	0.01	„	„	0.02	0.15	0.25	1.5	4.63
5. Control	—	„	0.15	0.43	2.8	14.2	29.2	90.1

The results in Table II clearly indicate the marked protection of these four ascorbyl esters of fatty acids on the storage of butterfat at 37° and as such, should find practical

application. The vitamin activities of these esters were checked by biological assay as detailed below.

Biological Availability of l-Ascorbyl Esters of Fatty Acids.—As the structure to which the antiscorbutic action of *l*-ascorbic acid is attributed might presumably have remained unchanged in these esters, it would be reasonable to believe that these fatty acid esters of *l*-ascorbic acid will also possess such activity. The object of the present work is to see how far this activity has been retained (or destroyed) in these esters. This can best be tested by determining the biological availability by subjecting them to biological assay with guinea-pigs.

Normal and healthy male guinea-pigs (av. bodyweight, 250-300 g.) were divided into 5 different groups; they were maintained on the following scorbutic diet consisting of crushed barley (64 parts), crushed gram (20 parts), casein (12 parts), cod liver oil (2 c.c.) and water to drink *ad lib*. When the animals just began to show a steep fall in body-weight (after 20-23 days) the first group of animals was given 0.5 mg. of synthetic *l*-ascorbic acid per animal. The 2nd, 3rd and 4th groups of animals were given an amount of the different esters containing 0.5 mg. equivalent of indophenol-reducing substance as a supplement to the above basal diet. The fifth group was used as a control to which no supplement was added to the scorbutic diet. The supplement was continued for two weeks when the animals showed steady and proportionate increase in body-weight. The results of the biological assay are shown in Table III below and graphically in Fig. 1.

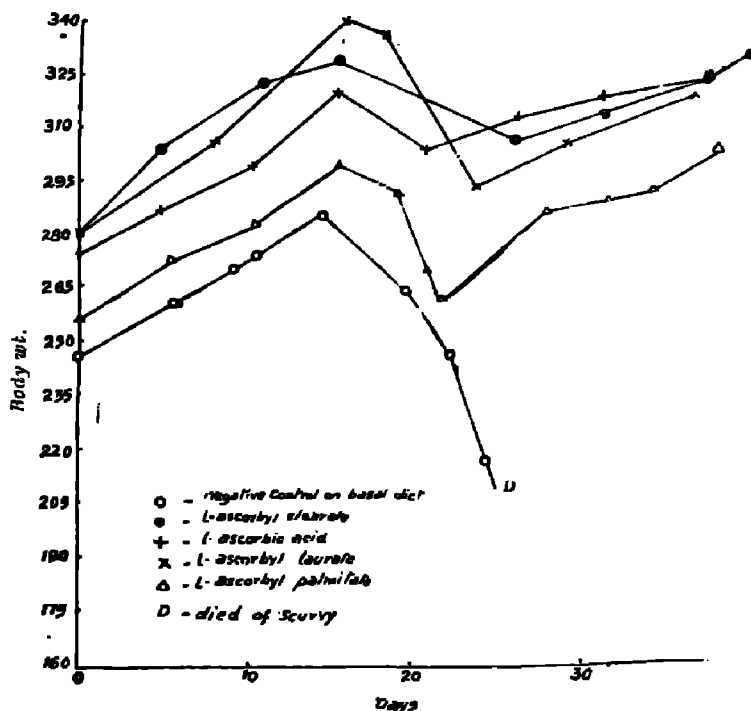
TABLE III

Group.	Daily supplement.	No. of guinea-pigs.	Average wt. on the day on which supplements started.	Average wt. after two weeks of supplement.	Average gain.
1	<i>l</i> -Ascorbic acid	2	296 g.	323 g.	+27 g.
2	<i>l</i> -Ascorbyl laurate	3	287	315	+28
3	„ palmitate	3	258	286.5	+28.5
4	„ stearate	3	310	335	+25
5	No supplement to basal diet	2	—	Died of scurvy	

These results clearly indicate that the ascorbyl esters possess antiscorbutic activity comparable to equivalent amounts of *l*-ascorbic acid. Hence, the use of such esters as antioxidants for fats provide a method for incorporation of vitamin-C in fats and oils. The biological utilisation of the fatty acid mono-esters of *l*-ascorbic acid being comparable to that of ascorbic acid itself, it would be advantageous in certain vitamin preparations to use this fat-soluble form of vitamin-C.

Of the esters tested *l*-ascorbyl palmitate was found to have the greatest activity, nearly 2.35 mg. of the esters being equivalent to 1 mg. of *l*-ascorbic acid.

FIG. 1



Antioxidant Losses during the Induction Period of Fat Oxidation.—It has been known for long that the part played by antioxidants in stabilising oils and fats is primarily to retard the oxidation of the unsaturated glycerides by inhibiting the normal chain of oxidation reactions that would have taken place in absence of the added antioxidants, these substances instead are themselves oxidised. It is expected therefore that the concentration of the effective antioxidant should show a decrease during the induction period of fats, and it seems desirable to determine to what extent such antioxidants, as *l*-ascorbyl esters of fatty acids, would be affected, for this will enable us to know the exact picture when such esters are actually incorporated in fats for stabilisation and fortification as well. For this purpose the loss of the *l*-ascorbyl esters during development of rancidity in butterfat was determined progressively. The antioxidants were added in two different concentrations of 0.05% and 0.02% in the butterfat and the samples oxidised at 37° by drawing in a slow current of purified and dried air through them at a constant rate. Samples were drawn out every 24 hours and determination of the peroxide content and *l*-ascorbyl esters was made, using a modified Wheeler method in the first case (Wheeler and Pascheek, *Oil & Soap*, 1944, 21, 33) and in the second, that of Turner and Speck (*Ind. Eng. Chem. Anal. Ed.*, 1944, 16, 464) using a standard solution of the sodium salt of 2:6-dichlorophenol-indophenol in dry acetone. The results are tabulated below,

TABLE IV

Disappearance of antioxidants during oxidation of butterfat at 37°

A. Peroxide value after days.

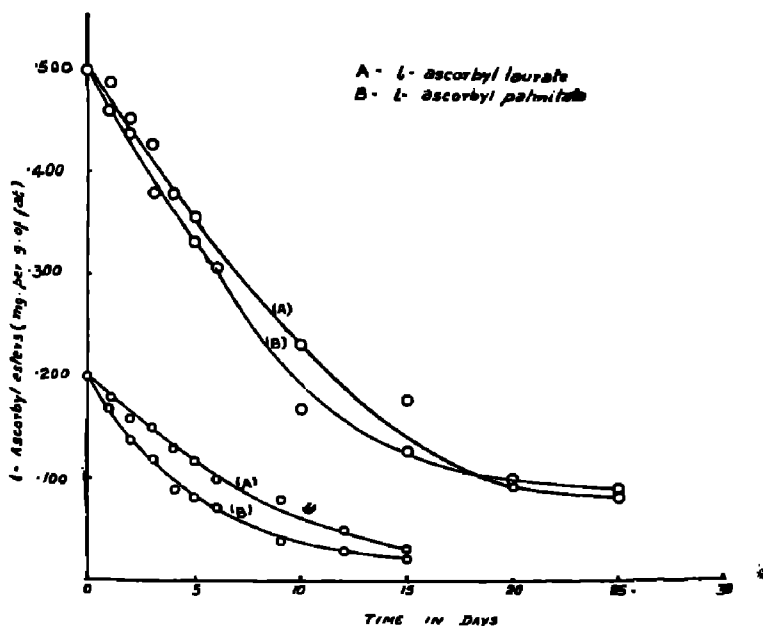
Antioxidants.	Initial conc.	1.	2.	3.	5.	6.	9.	10.	15.	20.
1. <i>l</i> -Ascorbyl palmitate	0.05%	0.0	0.1	0.5	1.0	1.5	2.6	4.3	7.0	10.2
2. " "	0.02	0.0	0.1	0.6	1.2	1.6	2.8	5.0	8.1	11.6
3. " laurate	0.05	0.0	0.14	0.4	0.9	1.2	2.0	4.0	7.0	10.2
4. " "	0.02	0.0	0.15	0.45	1.0	1.6	2.4	4.0	7.7	10.8
5. Control	—	0.2	0.8	1.2	4.0	6.75	8.6	16.5	18.2	20.3

B. *l*-Ascorbyl esters in mg./g. of fat after days.

		1.	2.	3.	4.	5.	6.	9.	10.	12.	15.	20.	25.
1. <i>l</i> -Ascorbyl palmitate	0.05%	0.49	0.45	0.43	0.38	0.36	3.32	—	0.14	—	0.10	0.095	0.085
2. Do	0.02	.18	.16	.15	.13	.12	.10	.08	.05	.05	0.3	—	—
3. <i>l</i> -Ascorbyl laurate	0.05	.46	.44	.38	—	.33	.31	—	.17	—	.13	.10	.09
4. Do	0.02	.17	.14	.12	.09	.08	.07	.04	—	.03	.02	—	—

Figure 2 represents the rate of disappearance of *l*-ascorbyl palmitate and laurate during the accelerated oxidation of butterfat at 37°. From Table IV the loss of *l*-ascorbyl esters during autooxidation appears to proceed at first fairly rapidly and then slowly within the induction period, but even after the induction period is over the whole of the ascorbyl esters are not destroyed. Moreover, a sharp rise in the peroxide concentration never occurs until more than half of the ascorbyl esters is destroyed, *e g.*, after 10 days of blowing.

FIG. 2

Rate of disappearance l-ascorbyl esters during oxidation at 37°.

The present study on the antioxidant behaviour of *l*-ascorbyl esters of fatty acids with butterfat reveals that in actual storage experiments, these esters retard the onset of rancidity to the extent that the induction period of the fat is more than doubled. These fat-soluble esters of ascorbic acid retain their antiscorbutic action and hence, when added as antioxidants, may serve as a source of additional vitamin. The loss of *l*-ascorbyl laurate and palmitate within the induction period has been determined, but it has been found that even when the induction period is over, the ester is not completely oxidised. A suitable method for the preparation of the various esters in suitable yield has been presented.

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