XX. THE INFLUENCE OF STORAGE ON THE ANTISCURVY VALUE OF FRUITS AND VEGETABLE JUICES.

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In recent years, attempts to place our knowledge of food values on a wider scientific basis have led to a series of researches on the effects of cold storage on a variety of products. One of the earliest of these was an investigation started in 1918 at the Lister Institute which was organised by Dr H. Chick and assisted by a grant from the Food Investigation Board. The object of the experiments was to find out the effects of prolonged storage under various conditions on certain common fruit and vegetable juices known to be of considerable antiscorbutic value when fresh. The tests have been carried out from time to time by a number of workers, all following the same methods and with a considerable amount of co-operation. Preliminary results with chilled fruits and fruit juices stored at various temperatures were published by Davey from the Lister Institute in 1921. The continuation of the work was interrupted however owing to unforeseen circumstances and the results have not yet been presented in a collected form. The research begun at the Lister Institute by Davey and others was continued by me in 1923 at the Biochemical Department, Institute of Physiology, University College and in the following year at Westfield College, in the Department of Botany, with the help of a grant from the Food Investigation Board. I have to thank Prof. Drummond for his kindness in connection with the experiments at University College and Dr H. Chick for giving me free access to previous records at the Lister Institute and for kindly reading the proofs of this article.

The technique of testing the juices is that described in detail by Delf and Tozer [1918], following in the main that of Chick, Hume and Skelton [1918]. Young guinea-pigs weighing at first about 320 g. were fed on a basal diet of crushed oats and bran together with a ration of 60 to 90 cc. of cow's milk previously autoclaved at about 120° for one hour. The fruit juices to be tested were fed by hand as daily doses and the smallest dose which effected protection from scurvy over an experimental period of 90 days was taken as the minimal protective ration for comparative purposes.

I. Cold storage of fruits and vegetables or their juices.

(*a*) *The storage of chilled fruits and vegetables.*

Oranges, lemons, tomatoes and swede-turnips were selected for trial as being easy to obtain in quantity and also rich in their content of vitamin C. Only carefully selected sound material was used. Each specimen was wrapped in paper to prevent contact with its neighbour, packed in a crate and conveyed at once to a chilling room of the Union Cold Store, Red Bull Wharf, E.C., whose proprietors kindly placed a chamber in their establishment at the disposal of the Lister Institute for this research.

(1) *Oranges.* A number of selected Denia oranges were packed and placed in the chilling room (temp. 2·5–5·4°) on May 7, 1919. They were inspected in July and again in October the same year when feeding experiments were started. At this time 96 oranges were still available of which only 11 were sound. The worst fruit was used first, the more rotten parts being cut away and the juice of the remainder squeezed as required through muslin. It was shown by Davey [1921] that the minimum protective dose of this juice was equal to that of the fresh raw fruit, being only 1·5 cc. daily. The storage and partial decay of the fruit had thus not appreciably impaired the antiscorbutic value of the juice.

(2) *Lemons.* Lemons packed and placed in the chilling room about the middle of April 1919 were inspected in July and November of the same year and were finally removed and tested in January 1920. Their condition was worse than that of the oranges, none being sound at the beginning of the experiment. The juice of these was fed to the experimental animals without previous neutralisation and frequently unsound parts of the lemons were used so that the juice was often discoloured. The daily doses were 1·5 cc., 2·5 cc. and 5 cc. respectively. In every case severe scurvy resulted, 51 days being the longest time of survival; little if any protection was therefore received.

The conclusion drawn from these experiments was that the juice of oranges and probably also of lemons does not lose appreciably in antiscorbutic value as long as the fruit is fit for food, but that the fruit cannot be kept in the chilled condition for more than four or five months.

(3) *Swede-turnips.* On May 10, 1919, swede-turnips were procured, some from Covent Garden and some from Newcastle through the kindness of Prof. Hutchens. Each consignment was examined immediately on arrival, only sound roots being used for the chilling room. The swedes were inspected in July and again in October of the same year. On the latter date, only a dozen of the Covent Garden batch remained sound; those from Newcastle were all more or less rotten: a few however were brought to the Lister Institute and tests were begun on the following day. They were only continued for 50 days owing to the rapid decay of such swedes as remained and towards the end of the experiment juice from unsound parts had to be included in the doses fed to the animals. Two animals on a dose of 2·5 cc. developed severe scurvy, this amount being

a sufficient ration in the case of the fresh juice. Two animals, each receiving 5 cc., were chloroformed after 35 days owing to lack of further juice. One showed typical scorbutic lesions while the other was normal. This experiment is inconclusive with regard to the effects of "chilling" since on the second visit to the store, some of the roots were found frozen which probably accounted for the rapid decay of the tissues. It is also inconclusive with regard to the antiscorbutic value of the juice, since protection was not attained over any appreciable experimental period. However, the results are not inconsistent with the conclusion derived from the consideration of the chilled oranges and lemons.

(4) *Tomatoes.* On August 6, 1919, a similar attempt was made to preserve tomatoes in the chilling room of the Union Cold Store. These were in fine condition when taken; they were examined in October the same year, and seemed mostly sound; a few with dark spots were carefully isolated with folds of paper. Examined again in January 1920, all the fruit was found to be spoiled and no further attempt was made to repeat the experiment.

(*b*) *Storage of fruit and vegetable juices.*

For commercial purposes, fruit juices are often preserved by the addition of a small proportion of some substance such as potassium metabisulphite. At low temperatures, however, the juice may be kept without the addition of any preservative and early in 1919, experiments were started to determine the influence of prolonged cold storage on the juice of oranges, lemons and swedes respectively.

(1) *Lemon.* On March 27, 1919, a number of sound lemons were well washed, dried and squeezed, the juice being then passed through muslin and placed in small glazed earthenware jars of about 150 cc. capacity, which were then at once corked and sealed with wax. A number of these jars were immediately stored in a cold chamber of the Colonial Consignment and Distributive Co., Ltd., which was generously set aside by them for the purpose. The temperature was checked by means of an automatic recording instrument and only varied from -11 to $-14°$. When samples of the juice were withdrawn for testing, they were always found to be frozen.

This juice remained undisturbed until August 1920 when test experiments were started at the Lister Institute by Davey, a jar of the frozen juice being fetched every fortnight for the purpose. Two animals each receiving a daily ration of 1·5 cc. of this juice remained free from scurvy for 90 days. This is no more than the minimal daily amount of the fresh juice giving protection from scurvy; there was thus no appreciable loss of vitamin during the first 17 months of cold storage.

In February 1924 some of the remaining juice was tested, jars of juice being fetched once a week. Three animals each receiving 1·5 cc. of the juice early began to show typical symptoms of scurvy. When these became severe, the ration was increased to 2·5 cc. One showed no improvement and was

chloroformed after 38 days; the other two were given a further allowance (2·5 cc. for 12 days, 3 cc. for the last 51 days). At the post-mortem, all these showed typical scorbutic lesions, severe in the first case, and nearly healed in the case of the two on increased doses. Three other animals on a daily ration of 3 cc. throughout the experiment remained in perfect health and showed no sign of scurvy at post-mortem examination.

These experiments show clearly that during five years' storage in the frozen state, a slow deterioration takes place, a minimum ration of nearly 3 cc. being required for protection instead of 1·5 cc. the amount needed of the raw fresh juice. Of the 135 jars originally stored only 22 have been used, the rest being still buried under an accumulation of snow in the freezing chamber. By prolonging the curve B (Fig. 1) smoothly, it will be seen that if the deterioration continues in the same way, a dose of about 5 cc. given in 1926 (*i.e.* after seven years' storage) should just suffice for protection.

(2) *Orange.* On March 13, 1919, orange juice was prepared, sealed and taken to the same cold storage chamber as the lemon juice. The first tests were made by Davey beginning in August 1920. As in the case of the lemon juice, a daily dose of 1·5 cc. was found to give complete protection and no measurable deterioration had occurred after 17 months of cold storage.

In February 1924 further tests were carried out by the writer, after five years of storage. Three animals on the usual basal diet were each given daily doses of 1·5 cc. of the juice. After 40, 42 and 49 days respectively, symptoms of scurvy became defined; the doses were then increased until at the end of the whole period the average ration per day amounted to 2·5 cc. At post-mortem examination all were found to have enlarged rib-junctions and dark coloured muscles; and one had also small lesions in the tissues around the knees and rib-junctions.

Three other animals kept on a uniform ration of 3 cc. survived for 91 days in good condition, but with occasional symptoms of scurvy. At the post-mortem examination, two had somewhat brittle bones, with some of the rib-junctions beaded; the third had in addition small petechial haemorrhages over the rib-junctions of both right and left side. This dose may therefore be taken as marginal in value. From these results, it appears that this sample of orange juice is perhaps somewhat less stable than the lemon (Fig. 1, A, B).

(3) *Swede-turnip.* The swedes for this experiment were obtained, some from Covent Garden and some from Newcastle as for the storage of the whole roots by chilling. The swedes were washed, grated on an ordinary kitchen grater, and the resulting pulp squeezed through muslin. The juice was placed in small glazed earthen jars of about 70 cc. capacity and was stored with the orange and lemon juice on 14th May 1919.

In August 1920, after 15 months' storage, the frozen juice was tested at the Lister Institute by Davey. Two animals each receiving 2·5 cc. of the juice developed acute scurvy and were chloroformed in an advanced condition after 37 days. Two others each receiving 5 cc. developed symptoms of

scurvy on the 26th and 28th days respectively. Both were chloroformed after 35 days of experiment and the post-mortem examination showed typical signs of severe scurvy. Two other animals were given 10 cc. daily beginning in October 1920; both became very ill with scurvy complicated by intestinal trouble, and had obviously received no protection. The administration of larger doses of the juice was not practicable.

Swede juice thus differs materially from the juice of oranges and lemons in that after only 15 months of cold storage in the frozen condition practically no antiscorbutic value remains. Some of this juice remains still in the freezing chamber and might be worth comparison with the fresh juice from the chemical point of view.

Table I. *The relative antiscurvy value of fresh and stored frozen juices.*

	Minimum protective ration cc.	Loss of vitamin C
Orange, fresh, raw	1·5	
Stored, frozen:		
1 year 5 months	1·5	Not appreciable
5 years	>2·5	More than two-fifths
	about 3	About half
Lemon, fresh, raw	1·5	
Stored, frozen:		
1 year 5 months	1·5	Not appreciable
5 years	>2·5	More than two-fifths
	<3	Less than half
Swede, fresh, raw	2·5	
Stored, frozen:		
1 year 3 months	> 5	Much more than half
1 year 5 months	>10	„ „ three-quarters

In the series of experiments described above, uniformity of material was secured as far as possible by the choice of ripe sound fruit, and by testing samples of the same material before and after storage. This is of some importance, since it is known that the antiscurvy value of the fresh product is itself subject to a good deal of variability. It has been proved for instance that the antiscorbutic value of orange juice diminishes somewhat towards the end of the season [Davey, 1921, p. 89], and that young carrots are more effective than old ones [Hess and Unger, 1919]. How far these and other variations in the condition of the fruit also affect the keeping properties is almost entirely unknown. Recent researches on the cold storage of apples show that in at least one variety, the time of survival in cold storage varies greatly, according to the age of the tree, the orchard and the season in which the crop was grown [Haynes, 1924].

The striking contrast between the behaviour of orange or lemon and swede juice calls for further comment. An obvious difference lies in the acidity. Acid fruit juices are found to be considerably more stable than neutral ones as regards antiscorbutic properties. Recently Zilva [1923, p. 416] has found that when decitrated concentrated lemon juice has part of its acid restored

there is much less loss of antiscorbutic properties on storage. According to La Mer and Campbell [1921] and La Mer [1921] the degree of acidity in tomato juice also affects its stability when exposed to rise of temperature. However, different juices of about the same natural acidity may have very different degrees of stability in their antiscorbutic properties, *e.g.* swede juice is more stable to heat than cabbage juice, and orange juice nearly neutralised as tested by Delf [1920] is much more stable than tomato juice neutralised to about the same extent as described by La Mer [1921].

In the preparation of the orange, lemon and swede juice for storage, no attempt was made to exclude air and slow oxidation doubtless occurred. Onslow has recently shown that in many fruits (apple, plum, pear) the cell sap contains enzymes and their substrates forming an autoxidising system which is incomplete in the case of orange, lemon and various other acid fruits. It is possible therefore that the greater destruction in the swede juice may be due to some kind of autoxidation in the juice, but there is no direct evidence as yet upon this point.

II. Storage of canned fruits (oranges and tomatoes).

(*a*) *Oranges.* In April 1919, a number of Denia oranges were peeled, divided into convenient parts and packed into cans[1]. These were processed for 20–30 minutes, the temperature rising gradually to 100° and remaining at 100° for not more than five minutes. The cans were then sealed, cooled and stored at laboratory temperature. Five months later, the first test was made. Allowing for the small amount of water added to the fruit after packing the cans, a dose equivalent to 1·5 cc. of the fresh juice was found to give adequate protection from scurvy. No loss of antiscorbutic activity was therefore indicated at this time.

A further test was made after four years' storage, beginning on June 6, 1923. Three animals each receiving daily 3 cc. of the juice escaped from scurvy, but at post-mortem examination the muscles were of a very dark colour, and in two cases small periosteal haemorrhages were seen in the regions of the costochondral junctions. These doses were measured without allowance for the water added in canning and represent 1·5–1·7 cc. of the original juice. Animals on a higher ration (equivalent to 2·5 cc.) were completely protected from scurvy. The canned orange juice has thus lost but a small proportion of its original antiscurvy value, after storage for more than four years. If the minimum protective ration be plotted against the time of storage, it appears that the canned juice stored at laboratory temperature is more stable than the raw juice kept at − 10° to − 14°. In view of this result, it seems probable that swede juice could also be successfully stored if previously heated and sealed in absence of air. This has only a theoretical interest, but it would

[1] The first batch was prepared by Miss Campbell; the rest by myself; the testing was done by myself.

probably apply equally to fruits such as pears and plums which are frequently bottled but the antiscorbutic value of which is little known.

(*b*) *Tomatoes.* The effect of storage on canned English tomatoes has been recently described [Delf, 1924]. The minimum protective dose of the raw juice lies near 1·5 cc. (estimated at about 2 cc.); that of the freshly canned tomato was about 8·5 cc.[1], this deterioration being in marked contrast to that which occurred with orange juice. After storage for four years, a dose averaging 10 cc. daily gave almost perfect protection for 68 days. For the full experimental period of 90 days a slightly increased ration would probably have been needed. The deterioration caused by storage is slow but is greater than that in the case of heated orange juice (Fig. 1). The reason for this is not clear since both are acid fruits and were canned and stored in the same way. As a matter of fact, it was noticed that the tomato juice when removed from the tins on opening did not keep as well as the orange juice especially in the hot weather. For the most part, juice showing signs of fermentation was rejected, but owing to the small supply fresh tins of canned tomatoes could not be opened as frequently as desired especially towards the end of the experiment.

Table II. *The relative antiscurvy value of freshly canned and stored canned fruit.*

	Minimum protective ration	Loss
Orange juice:		
Fresh, raw	1·5 cc.	
Canned, newly	1·5 cc.	Not perceptible
Canned and stored 4 years at lab. temp. ...	1·7 cc.	About 10 %
Tomato:		
Fresh, raw	1·5–2·5 cc.	
	Estimated at about 2 cc.	
Canned, newly	7·5–10 cc.	
	Estimated at about 8·5 cc.	About 75 %
Canned and stored 4 years at lab. temp. ...	>10 cc.	Over 80 %

III. Storage of raw lemon juice with added preservative.

In 1918 a quantity of lemon juice was prepared for storage by the addition of potassium metabisulphite as described by Davey [1921].

(*a*) *Material preserved with* ·06 % *sulphite.*

Two lots of this material were made up. The first made in February 1918 was tested after four and again after six months, but the results were not satisfactory owing apparently to exposure to air during the intervals of the experiment, and are set aside for the purpose of the present discussion. A further test on the second lot of material was made at intervals varying from 18 to 22 months. The experiments were not quite finished at the time

[1] According to Hess [1921] and La Mer [1921] bottled tomato juice in America has the much lower minimum protective ration of 3 cc.; the juice used by La Mer was prepared by a well-known canning company and was processed for a much shorter time than that prepared and used at the Lister Institute.

when the account by Davey went to press, but apparent protection was given by doses of 2·5 and 5 cc., no post-mortem examination being made in either case. In 1923 a further test was made by me at University College. No special precautions were taken to exclude air from the stock bottle which was kept at laboratory temperature throughout the experiment. No deterioration seemed to occur either in the appearance or taste during this time.

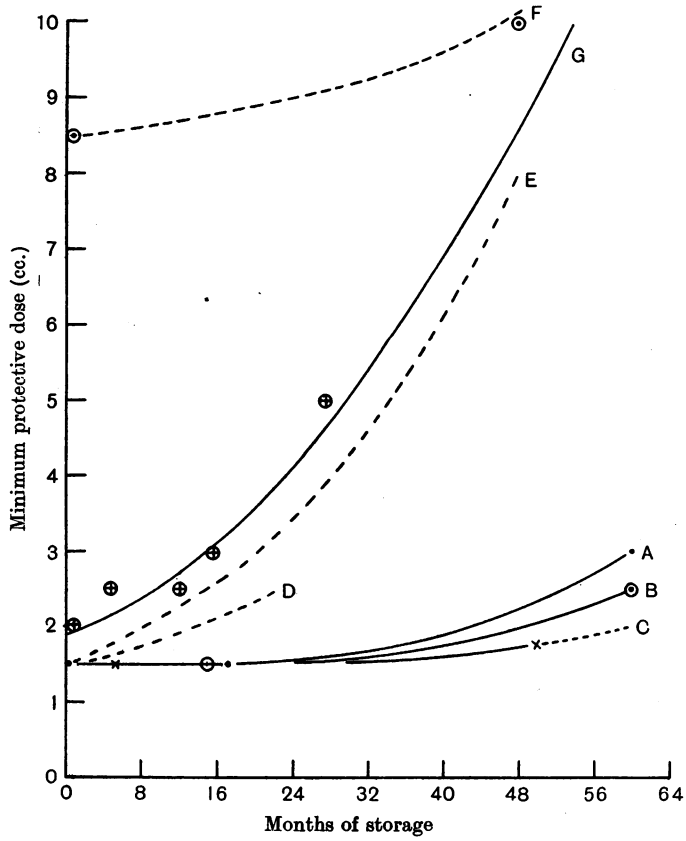

Fig. 1. Diagram showing relation between time of storage and minimum protective ration.

(A) Frozen orange juice (·—·), data as in Table I.
(B) Frozen lemon juice (⊙——⊙), data as in Table I.
(C) Canned orange juice (×—×), data as in Table II.
(D) Sulphite lemon juice ·06 % (cold room), data as in Fig. 2, D.
(E) Sulphite lemon juice ·06 % (lab. temp.), data as in Fig. 2, E.
(F) Canned tomato juice (⊙- - - - - -⊙), data as in Table II.
(G) Lemon juice with rind oil (lab. temp.) (⊕ — ⊕), data as in Table III.

Note. The curves D and E are taken from those in Fig. 2 of the same lettering, where the amount of approximation is shown on the same scale.

Three strong young guinea-pigs were selected for the purpose of the test. They disliked the juice so much that for the first 12 days the ration was kept

at 5 cc., after which it was increased to 10 cc.[1] fed in two daily instalments with other food between. The animals made fairly good growth and escaped typical scurvy but often showed signs of tenderness in the hind limbs. At post-mortem examination, after 88 days of experimental diet, one of the three was found to be quite normal; one had a haemorrhage into the wall and cavity of the middle region of the small intestine and the third had two small periosteal haemorrhages near the costochondral junctions. In every case the lower molars and in one case the tibiae also were markedly brittle. This dose, averaging 9 cc. daily over the whole period, therefore gave apparent protection, which, as seen from the post-mortem, was very nearly marginal. The undoubted value of the juice in this case suggests that this sulphite sample stored at laboratory temperature had a considerable degree of stability. It is obvious, however, that it is inferior in this respect either to frozen juice (Fig. 1, cp. E, B) or to the same juice stored at a lower temperature (Fig. 1, D).

A sample of this preparation was also stored at about + 4° and was examined after 27 months [Davey, 1921].

The results of these and other experiments are collected in Table III. Assuming the process of ageing to be an orderly one and making allowances for the approximate nature of some of the results, they may be expressed graphically (Fig. 2). The general form of the curve E is defined by the determination of the minimum dose after 4½ years' storage; the other points are less well known, but unless there was any initial rapid loss of antiscorbutic value on adding the preservative (a point which was not investigated at the time) the course of deterioration might be expected to follow some such direction as the line suggested in the smoothed curve E. The curve F is not so well defined since the final test failed to give protection; from what has been said about the relative value of the other doses, it cannot be very different from that indicated and the continuation of it must at least indicate a rate of deterioration considerably greater than that of the juice with a smaller amount of added preservative (curve E). This is clearly in opposition to the tentative conclusion of Davey [1921, p. 98] that the larger amount of preservative was probably to be recommended.

(*b*) *Material preserved with* 0·09 % *sulphite.*

Results of the earlier experiments with juice having a final concentration of 0·09 % added sulphite were briefly indicated by Davey [1921], but the experiments were unfinished at the time of publication. After seven months' storage, a daily dose of 5 cc. gave protection but no lower dose was then tried. After 12 months, three animals on a dose of 2·5 cc. received partial protection, symptoms of scurvy becoming well defined but not developing until the end of the experiment. The three animals on this dose behaved differently, one

[1] The average dose for the whole period was 9 cc., but since the lower ration was fed only at the beginning of the experiment, the result may be taken as due to a ration throughout of about 10 cc.

dying with severe scurvy after 61 days, another showing no symptoms until after 66 days and the third having no symptoms until after 94 days. In the case of the last animal the scurvy was of a definite but mild type although the experiment was prolonged for 100 days.

After 19 months' storage, a daily dose of 5 cc. gave considerable protection to each of two animals. The first developed symptoms after 78 days and died with severe scurvy after 84 days of experiment; and the second developed symptoms after 84 days and survived for 90 days, showing signs of mild scurvy at post-mortem. A dose of 10 cc. afforded complete protection in an experiment begun when the juice was 22 months old and continued for 86 days.

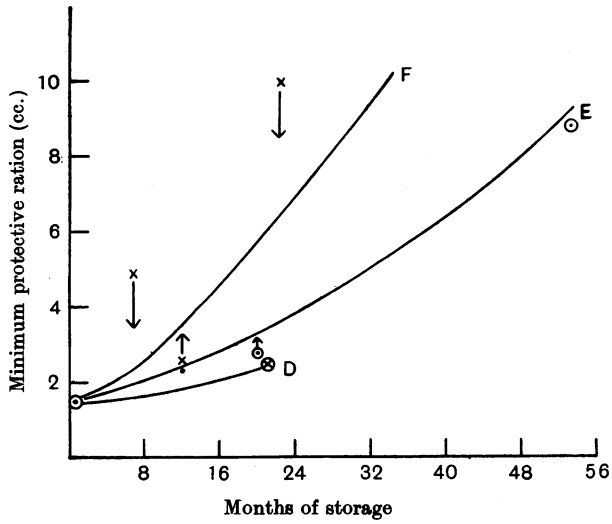

Fig. 2. Diagram showing the relation between time of storage and probable minimum protective ration; lemon juice with added sulphite:

·06 % stored at laboratory temperature, (⊙ ⊙) E.
·06 % stored in cold room (4–10°), (⊗ ⊗) D.
·09 % stored at laboratory temperature, (× × ×) F.

In June 1923 an attempt was made by me to apply a further test, after $4\frac{1}{2}$ years of storage at laboratory temperature. Three animals were started on a daily dose of 5 cc., but owing to difficulties of feeding the ration was not further increased. Symptoms of scurvy were noticed on the 52nd, 53rd and 67th day respectively. The symptoms were progressive, the last animal surviving for only 80 days, when it was chloroformed in an advanced stage of severe scurvy. No larger doses were practicable, but a consideration of all the results seems to show that the higher percentage of sulphite is not conducive to the preservation of the antiscorbutic value of the juice (cp. Fig. 2).

IV. The use of rind oil as a preservative.

The fresh juice of lemons and oranges as prepared with the rind oil by Davey in 1918 was stored part at laboratory temperatures[1] and part in the cold room of the Lister Institute. The results are given by Davey [1921] and are summarised for reference in Table III. In the summer of 1923, a further test was made using a daily ration of 5 cc. This gave partial protection to each of three animals which developed "chronic" scurvy, becoming severe but not fatal. It was impossible to feed larger doses at the time but some indication of the course of deterioration may be seen by plotting all the values found (Fig. 1, G).

Table III. *Lemon juice with added preservative.*

Condition	Temp.	Age	Minimum protective dose	
Raw	Laboratory	Fresh	1·5 cc.	
With ·06 % sulphite	,,	18–22 months	? >2·5–<5 cc.	Fig. 2, E
	,,	4½ years	9–10 cc.	Fig. 2, E
	Cold room	27 months	<5 cc.	Fig. 2, D
			>2·5 cc.	Fig. 2, D
With ·09 % sulphite	Laboratory	7 months	< 5 cc.	Fig. 2, F
		12	> 2·5 cc.	Fig. 2, F
		19	> 5 ,,	Fig. 2, F
		22	<10 ,,	Fig. 2, F
		4½ years	> 5 ,,	Fig. 2, F
With rind oil	Laboratory	Fresh	>1·5<2·5 cc.	Fig. 1, G
		4½ months	<2·5 cc.	Fig. 1, G
		12	<2·5 ,,	Fig. 1, G
		27	5 ,,	Fig. 1, G
		5 years	>5 ,,	Fig. 1, G

A similar set of experiments over a shorter period was carried out using orange juice. The stored orange juice behaved in much the same way as the lemon juice [Davey, 1921] but there is not sufficient evidence to make a detailed comparison of the stability of the two juices.

General conclusions.

(1) When whole fruits (oranges or lemons) or tuberous roots (swedes) are stored, their antiscorbutic value is retained as long as the tissue itself remains living.

(2) The expressed juice of oranges and lemons stored for five years in the frozen condition retains about half its original antiscorbutic potency. Swede juice on the other hand stored in a similar way, loses the greater part of its value in less than two years. It is suggested that the greater deterioration in the latter case may be due to the lower acidity or to autoxidation or to both.

(3) Canned orange and tomato juice retain much of their value even after storage for four years at laboratory temperature. The tomato as prepared for these experiments was much less stable than the orange.

[1] For the first 10 months all the juice was kept in the cold room; later part was removed and stored at laboratory temperature.

(4) Lemon juice with added potassium bisulphite retains its flavour well after storage for $4\frac{1}{2}$ years at laboratory temperature. At this age, the protective dose of lemon juice containing ·06 % sulphite was about 10 cc. instead of the original 1·5 cc., thus indicating a loss of about five-sixths of the original value.

(5) Both orange and lemon juice can be preserved satisfactorily for one to three years at laboratory temperature in presence of their own rind oil.

(6) Inspection of the comparative curves in Fig. 1 shows the relative stability of the juices examined. Canned orange is the most stable and this is in agreement with previous findings with heated juices [Delf, 1920]. The frozen juices are next in order of stability; the form of the curves in preserved juices suggests that the added sulphite has a destructive effect on the stability of the antiscorbutic vitamin.

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