V. THE PRESERVATION OF THE ANTI-SCORBUTIC VITAMIN IN LEMON JUICE.

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I. The preservative action of potassium metabisulphite and lemon rind oil.

The first work on the preservation of the antiscorbutic vitamin was performed by Davey [1921], who found that lemon juice could be preserved satisfactorily at a low temperature (about 0°) by both potassium metabisulphite and lemon rind oil respectively. At laboratory temperature preservation was unsatisfactory in the case of metabisulphite, but reliable in that of rind oil. At 37°, neither rind oil nor metabisulphite was satisfactory. An interesting result of Davey's work lies in the fact that apparently 0·09 % metabisulphite is less satisfactory in its preservative effects on the antiscorbutic vitamin than is 0·06 % metabisulphite. This suggests that metabisulphite (or actually sulphur dioxide) has a destructive effect on vitamin C. Delf [1925] states that lemon juice containing added metabisulphite retains its flavour well after storage for $4\frac{1}{2}$ years at laboratory temperature, but that in the case of juice containing 0·06 % metabisulphite, only one-sixth of the original antiscorbutic potency remained after this time. Over a period of time, the preservative effects of 0·06 % metabisulphite and lemon rind oil respectively were similar, and were superior to that of 0·09 % metabisulphite.

A survey of the literature on the behaviour of the antiscorbutic vitamin shows that it is relatively stable in acid solutions, but is destroyed rapidly in alkaline solutions in the presence of air [Harden and Zilva, 1918; Hess and Unger, 1919]. Oxidation has a definite destructive action on the potency of active solutions of vitamin C [Hess and Unger, 1921; Zilva, 1921; Dutcher, Harshaw and Hall, 1921]. Zilva [1923] finds that, whilst in the presence of air vitamin C is rapidly destroyed in alkaline solution, in the absence of air destruction is prevented. More recent work by Zilva [1927] shows that the stability of the antiscorbutic vitamin runs parallel with the presence of a reducing factor which he estimates by titration with phenolindophenol in neutral solution. Whilst the vitamin and the reducing factor are two distinct substances, the reducing factor being destroyed more rapidly than vitamin C, he finds that in all cases there is a distinct parallelism between the two substances. In a later paper Zilva [1928] suggests that "the stability of the antiscorbutic factor in lemon juice is conditioned by the presence of a reducing

principle and of a factor, the functioning of which is destroyed by heat. ...The reducing property of the solution acted as a 'reduction buffer' for the antiscorbutic vitamin." "Although acidity does not prevent the destruction of this last factor" (the heat-labile) "by heat, it exercises definite protection of the reducing and antiscorbutic principles after anaerobic heating." Szent-Györgyi [1928] suggests that this reducing factor is identical with the hexuronic acid which he isolated from various juices and which plays an important function in peroxidase systems.

The present investigation is an attempt to develop the work initiated by Davey and Delf, and to discover the conditions necessary for satisfactory preservation of vitamin C in lemon juice.

Experimental.

All samples of lemon juice used in this investigation were stored (while in the laboratory) in well-stoppered bottles (previously sterilised in a steamer) at room temperature. The temperature was constant between 15° and 18° during the tests. After storage, the juices were administered in daily doses to young guinea-pigs of approximately 300 g. weight, kept on a basal diet of bran and oats with water *ad libitum*. Vitamins A and D were supplied daily in the form of cod-liver oil (0·05–0·1 g. daily per animal). On this basal diet the guinea-pigs grow well until the onset of scurvy. Immediately before administration, the juice was partly neutralised with calcium carbonate, a procedure which does not involve any destruction of the vitamin as was shown by a blank experiment. During the progress of the experiment, the characteristic symptoms of scurvy were noted and *post-mortem* examinations were carried out on each animal. Throughout this series of experiments the presence or absence of characteristic haemorrhages and beading of rib junctions was taken as the decisive factor in diagnosing scurvy.

As far as possible, the minimum protective dose of juice was used to define antiscorbutic potency. Where no actual determination of the minimum protective dose was made, the time taken to develop scurvy on any particular dose of juice was assumed to give a reasonable idea of the antiscorbutic potency. It is, of course, realised that this latter procedure is only approximate, since guinea-pigs vary considerably in the time taken to show symptoms of scurvy. When, however, three or more guinea-pigs, receiving doses varying between 1·5 and 3·5 cc., show symptoms of acute scurvy after 20–30 days, it is evident that the juice in question contains very little antiscorbutic vitamin.

Comparative effects of potassium metabisulphite and lemon rind oil.

Fresh lemons were cut in half and squeezed by hand on a glass squeezer. The expressed juice was filtered through fine muslin and was then kept in previously sterilised stoppered Winchester quart bottles. The required amounts of potassium metabisulphite and lemon rind oil were added to the respective samples as follows: 0·02 % potassium metabisulphite, 0·07 %, 0·275 %, 0·5 %

Table I. *Preservative action of potassium metabisulphite and lemon rind oil*

Sample	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions	Minimum protective dose (cc.)	General condition of juice
Lemon juice + 0·02 % metabisulphite	273	♀	1·0	23	Acute scurvy in each case. All the animals had to be killed after showing severe symptoms of scurvy. *Post-mortem* examinations showed extensive haemorrhages	3·5	Juice clear, golden-yellow colour, heavy growth of moulds on surface
	260	♀	1·5	19			
	290	♂	2·0	23			
	286	♀	2·5	25			
	281	♀	3·0	30			
	262	♀	3·5	25			
Lemon juice + 0·07 % rind oil	282	♂	1·0	27	Acute scurvy—severe extensive haemorrhages	2·5	Juice slightly cloudy and slight growth of moulds: much less than juice with metabisulphite
	271	♀	1·5	48	Acute scurvy—severe extensive haemorrhages, femora and costochondral junctions		
	268	♀	2·0	27	Acute scurvy—severe extensive haemorrhages, femora and costochondral junctions		
	279	♀	2·5	48	Slight haemorrhage on jaws: otherwise quite normal and healthy		
	265	♀	3·0	48	Slight haemorrhage on jaws: otherwise quite normal and healthy		
	283	♂	3·5	48	Abscess under jaw: otherwise quite normal and healthy		
Lemon juice + 0·275 % rind oil	266	♂	1·0	27	Acute scurvy—extensive haemorrhages	3·0–3·5	Juice slightly cloudy: no moulds
	274	♀	1·5	27	Acute scurvy—extensive haemorrhages		
	258	♀	2·0	48	Scurvy—severe haemorrhages, femora, etc.		
	259	♀	2·5	27	Acute scurvy—extensive haemorrhages		
	285	♀	3·0	48	Slight haemorrhages on jaws: otherwise quite healthy		
	269	♂	3·5	48	Spot of haemorrhage on femora and jaws: otherwise quite healthy		
Lemon juice + 0·5 % rind oil	267	♀	1·0	19	Acute scurvy—extensive haemorrhages	3·0–3·5	Juice clear: no moulds
	276	♀	1·5	40	Acute scurvy—extensive haemorrhages		
	280	♂	2·0	48	Scurvy		
	264	♀	2·5	23	Acute scurvy—extensive haemorrhages		
	278	♂	3·0	48	Haemorrhage on jaw and costochondral joint (slight)		
	275	♂	3·5	48	Slight haemorrhage on jaw: otherwise quite healthy		
Lemon juice + 1·1 % rind oil	268	♀	1·0	35	Acute scurvy—extensive haemorrhages	3·5	Juice clear: no moulds
	277	♀	1·5	39	Scurvy—slight haemorrhages, femora and costochondral junctions		
	289	♀	2·0	39	Scurvy—severe femoral haemorrhages		
	257	♂	2·5	39	Scurvy—severe femoral haemorrhages		
	261	♀	3·0	39	Scurvy—severe haemorrhages		
	284	♀	3·5	39	Severe haemorrhages, one femur, otherwise healthy		

and 1·1 % lemon rind oil. After storage for 2½ months at room temperature the juices were administered to young guinea-pigs in daily doses ranging from 1·0 cc. to 3·5 cc. (Table I).

The results in Table I indicate that lemon rind oil is a better preservative than 0·02 % potassium metabisulphite from the point of view of gross fermentation and mould growth as well as from that of antiscorbutic potency. Moreover, it is seen that although the juices preserved with 0·275 % rind oil and above are better preserved in the gross sense, they are if anything slightly less potent in vitamin C than the juice preserved with 0·07 % rind oil, *i.e.* one-sixteenth of the proportion of rind oil to juice normally present in the average fresh lemon.

Following on this, samples of lemon juice were obtained from Sicily, preserved with 0·04 % potassium metabisulphite and 0·07 % and 0·275 % rind oil to test the relative preservative action of rind oil and metabisulphite under commercial conditions. In addition, a further sample was sent over in a cask which had been fumigated with a weak sulphur fume (SO_2) immediately before the juice was introduced into the cask. This last sample contained no other preservative. With the exception of the juice preserved with 0·04 % potassium metabisulphite, all the samples had fermented as was evidenced by the escape of gas when the samples were opened. It is to be noted that 0·04 % metabisulphite is a satisfactory preservative against gross fermentation, whereas 0·02 % metabisulphite, as used in the previous experiment, is unsatisfactory. Table II summarises the condition of the various samples of juices on arrival.

Table II. *Condition of preserved juices.*

Sample of juice	Treatment	Condition
A	Preserved with 0·04 % metabisulphite	No fermentation: juice clear
B	„ „ 0·07 % rind oil	Juice cloudy: some fermentation
C	„ „ 0·275 % „	„ „
D	Cask sulphured	Juice cloudy and fermented

As preservatives against fermentation, rind oil and mere sulphuring of the cask are definitely inferior to 0·04 % potassium metabisulphite. Only in the case of 0·04 % potassium metabisulphite was a palatable juice obtained.

From the date of pressing until the commencement of the experiment about a month elapsed. During this time the juices were exposed to the treatment ordinarily undergone by commercial imported lemon juice. Doses ranging from 1·0 cc. to 3·0 cc. each of the four samples were administered to the test guinea-pigs (Table III).

From these results it will be seen that the findings of the laboratory experiment are confirmed under commercial conditions with respect to the effect of metabisulphite and rind oil on the antiscorbutic vitamin. Here again the juice preserved with 0·07 % rind oil appears to be slightly more potent in vitamin C than that preserved with 0·275 % rind oil. An interesting fact is found in connection with the potency of the sample kept in a cask which had

merely been fumigated with sulphur dioxide. Here the concentration of sulphur dioxide was such as to be practically undetectable. This juice had preserved a high proportion of its antiscorbutic potency, in contradistinction to the sample preserved with 0·04 % potassium metabisulphite. These facts show definitely that potassium metabisulphite tends to destroy the antiscorbutic potency of lemon juice; if not directly, by reacting with some factor on which the stability of vitamin C depends. That this latter explanation is probably the case will, it is hoped, be shown in a later paper when the effect of potassium metabisulphite on the loss of reducing power of lemon juice is reported.

Table III. *Preservative action of potassium metabisulphite and lemon rind oil.*

Sample	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions	Minimum protective dose (cc.)
A. Lemon juice + 0·04 % metabisulphite	395	♀	1·0	52	Acute scurvy—extensive haemorrhages	> 3·0
	329	♀	1·5	43	Acute scurvy—extensive haemorrhages	
	312	♂	2·0	60	Acute scurvy—extensive haemorrhages	
	320	♀	2·5	46	Acute scurvy—extensive haemorrhages	
	318	♂	3·0	74	Acute scurvy—extensive haemorrhages	
B. Lemon juice + 0·07 % rind oil	340	♀	1·0	24	Acute scurvy—extensive haemorrhages	2·0–2·5
	345	♀	1·5	98	Slight scurvy—slight haemorrhages, femur	
	347	♂	2·0	98	Very slight beading of rib junction. No haemorrhages	
	324	♀	2·5	98	Quite normal and healthy	
	327	♂	3·0	98	Quite normal and healthy	
C. Lemon juice + 0·275 % rind oil	351	♀	1·0	30	Scurvy—slight haemorrhages	2·5
	349	♂	1·5	99	Slight scurvy—slight haemorrhages	
	343	♀	2·0	87	Slight scurvy—liver and kidney diseased	
	346	♂	2·5	99	Quite normal and healthy	
	341	♀	3·0	99	Quite normal and healthy	
D. Lemon juice in sulphured cask	350	♂	1·0	98	Slight scurvy—slight haemorrhages	2·0–2·5
	333	♀	1·5	91	Slight scurvy—haemorrhages, femora, etc.	
	322	♀	2·0	98	Very slight scurvy—very slight haemorrhages	
	310	♀	2·5	98	Quite healthy	
	342	♀	3·0	98	Quite healthy	

It has been stated that, except in the case of the juice preserved with 0·04 % potassium metabisulphite, the samples of imported lemon juice had fermented and were rather unpalatable. Davey [1921] and Zilva [1924] have stated previously that the sugar in lemon juice may all be fermented without any appreciable destruction of the antiscorbutic fraction. This is the case in the samples of juice used in this experiment. Whilst potassium metabisulphite (or sulphur dioxide) is undoubtedly the best preservative of lemon juice from the point of view of fermentation, it tends to destroy the antiscorbutic vitamin.

The rate of loss of antiscorbutic potency of lemon juice preserved with 0·04 % *potassium metabisulphite.*

It has been shown above that at laboratory temperature, lemon juice preserved with 0·04 % potassium metabisulphite for approximately 1 month had lost at least 50 % of its antiscorbutic potency. It is of interest to determine how quickly the antiscorbutic potency of lemon juice preserved with metabisulphite is lost.

A three weeks' supply of lemon juice was pressed weekly, the pulp being removed by filtration through muslin. The requisite amount of potassium metabisulphite was added. Three lots of guinea-pigs were taken for this experiment and daily doses of 1·5, 3·0, and 4·5 cc. were given. At the end of the first week, the juice was administered to one lot of guinea-pigs and continued for a week. They received juice which had been preserved an average of 10–11 days. At the end of three weeks the second set of guinea-pigs received the juice; this was given for 1 week so that the juice they received had been preserved an average of 25 days. The third set of guinea-pigs received the juice after 5 weeks; this juice was preserved for an average of 39 days. By this means the loss of vitamin C potency in the juice was followed over a period of 39 days (Table IV).

Table IV.

Juice	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions
Preserved 11 days	451	♀	1·5	96	No scurvy—healthy
	452	♀	3·0	96	No scurvy—perfectly healthy
	453	♀	4·5	96	No scurvy—perfectly healthy
Preserved 25 days	455	♂	1·5	36	Acute scurvy—severe extensive haemorrhages and beading of rib junctions
	454	♂	3·0	72	Acute scurvy—severe extensive haemorrhages and beading of rib junctions
	456	♀	4·5	94	No scurvy haemorrhages: very slight beading of rib junctions: apart from digestive disorders quite healthy
Preserved 39 days	460	♀	1·5	24	Severe extensive haemorrhages and beading of rib junctions
	462	♀	3·0	29	
	461	♀	4·5	29	Acute scurvy

From Table IV it will be definitely seen that the loss of antiscorbutic potency of lemon juice preserved with 0·04 % metabisulphite proceeds with a measurable velocity. It is hoped to demonstrate in a later paper that this loss of vitamin potency is to a certain extent parallel with the loss in reducing power of lemon juice preserved under identical conditions. The fact that vitamin potency and reducing power are present together has been noted previously by Zilva [1927].

II. The effect of acidity on the preservation of vitamin C.

It is a generally established fact that vitamin C is more stable in acid solutions than in alkaline solutions [cf. Zilva, 1923]. Moreover, high acidity also inhibits the growth of most bacteria and moulds. It was thought, therefore,

that an addition of acid to lemon juice would serve as a preservative both for the antiscorbutic vitamin and also against gross fermentation. With this end in view, a sample of juice made up to p_H 1·6 with hydrochloric acid was imported from Sicily. On its arrival (about 1 month after pressing) the juice was examined and fed to test guinea-pigs (Table V).

Table V. *The preservative action of hydrochloric acid in lemon juice.*

Sample	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions
Lemon juice + 0·3 % HCl (p_H 1·6)	316	♂	1·0	92	No apparent scurvy—slight haemorrhages on jaw, otherwise healthy
	335	♀	1·5	92	No apparent scurvy—slight haemorrhages on jaw, otherwise healthy
	308	♀	2·0	92	No apparent scurvy—slight haemorrhages on jaw, otherwise healthy
	311	♂	2·5	92	Quite normal and healthy
	319	♂	3·0	92	Quite normal and healthy

From this table, lemon juice preserved for 1 month with 0·3 % HCl (p_H 1·6) was as potent as fresh lemon juice as an antiscorbutic. It was not preserved in the gross sense however. The juice on arrival was yellow, cloudy and fermented. This sample of juice was tested for its antiscorbutic potency after a further 12 months' storage and was found to have preserved its potency intact, 1·5 cc. being a protective dose for a guinea-pig.

It was next deemed desirable to ascertain whether there was an optimum acidity for the stability of the antiscorbutic vitamin, when kept under ordinary laboratory conditions, and whether the acidity of the juice could be increased still further to inhibit mould growth and fermentation, at the same time maintaining a palatable juice rich in the antiscorbutic vitamin.

Fresh lemons were squeezed and the juice obtained as described above. Samples were adjusted to p_H 1·8, 1·4, 1·0 and 0·6 with pure HCl, whilst one sample was made up to p_H 3·0 with alkali. These samples together with a sample of ordinary (unpreserved) lemon juice were kept at room temperature (15°–18°) for 7–8 months in sterilised bottles, at the end of which time they were fed in doses corresponding to 1·0–3·0 cc. to young guinea-pigs. Immediately before administration to the test animals, the various samples were brought to the same p_H, namely that of fresh lemon juice (p_H 2·2) with standard alkali (or acid in the case of the less acid juice), the dilution of the juice being allowed for in the calculation of the size of dosage (Table VI).

It is apparent from Table VI that vitamin C is destroyed both in the more acid solutions above p_H 1·8 and in the solution at p_H 3·0, though in the last case to a much smaller extent. In the solutions of p_H 1·4 and less it appears that the vitamin is completely absent.

On keeping these solutions it was noted that in addition to the precipitate of pulpy material which invariably shows itself in solutions of lemon juice, a more flocculent precipitate appeared. The bulk of this precipitate increased as the

Table VI. *The effect of acidity on the stability of vitamin C.*

Juice	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions	Minimum protective dose (cc.)	Condition of juice
Lemon	366	♂	1·5	23	Extensive and severe haemorrhages—acute scurvy	> 3·0	No mould growth or fermentation: the juices turned brown on keeping, possibly due to hydrolysis of the protein in the juice
juice +	371	♂♂	2·0	23	Extensive and severe haemorrhages—acute scurvy		
HCl	365	♂♂	2·5	23	Extensive and severe haemorrhages—acute scurvy		
p_H 0·6	368	♂♂	3·0	23	Extensive and severe haemorrhages—acute scurvy		
Lemon	362	♂	1·5	27	Severe haemorrhages—scurvy	> 3·0	No mould growth or fermentation: the juices turned brown on keeping, possibly due to hydrolysis of the protein in the juice
juice +	373	♂♀	2·0	23	Severe haemorrhages—acute scurvy		
HCl	378	♂♀	2·5	21	Severe haemorrhages—acute scurvy		
p_H 1·0	364	♀	3·0	25	Found dead—emaciated condition: slight beading of costochondral junctions, and haemorrhages—incipient scurvy		
Lemon	372	♂	1·5	21	Weak condition—no definite evidence of scurvy	> 3·0	No mould growth or fermentation: the juices turned brown on keeping, possibly due to hydrolysis of the protein in the juice
juice +	382	♂♂	2·0	24	Found dead: emaciated condition—very slight scurvy		
HCl	379	♂♂	2·5	21	Severe haemorrhages—acute scurvy		
p_H 1·4	376	♂♀	3·0	26	Severe haemorrhages—acute scurvy		
Lemon	386	♀	1·5	55	Killed. Fractured femur—no symptoms of scurvy	1·5–2	Slight growth of mould and darkening of juice
juice +	385	♀♀	2·0	90	Perfectly healthy—no scurvy symptoms		
HCl	369	♀♀	2·5	90	Perfectly healthy—no scurvy symptoms		
p_H 1·8	367	♀♀	3·0	90	Perfectly healthy—no scurvy symptoms		
Lemon juice	384	♀	1·5	76	Slight haemorrhage, costochondral junctions—no other scurvy symptoms	1·5–2	Heavy mould and alcoholic fermentation
with no HCl	383	♂	2·0	76	Slight haemorrhage, jaw—no other scurvy symptoms		
p_H 2·2	370	♂♀	2·5	76	Slight haemorrhage, jaw—no other scurvy symptoms		
	363	♂	3·0	62	Found dead: very emaciated condition—no symptoms of scurvy		
Lemon	374	♂	1·5	67	Extensive haemorrhages—acute scurvy	3·0	Heavy mould and alcoholic fermentation
juice +	389	♂♂	2·0	75	Found dead: very emaciated—no definite scurvy symptoms		
NaOH	380	♂♂	2·5	82	Severe haemorrhages—acute scurvy		
p_H 3·0	388	♂♀	3·0	84	No scurvy symptoms		

latter solutions developed a dark brown colour, the depth of which also varied with increase in acidity. This is possibly evidence of hydrolysis of the protein and carbohydrate of the juice. In these latter solutions no evidence of mould growth or fermentation was detected, whereas in the solutions at p_H 1·8 and 2·2 a slight growth of mould was noted. No great degree of discoloration took place in these two solutions. In the juice adjusted to p_H 3·0 mould growth and strong evidence of alcoholic fermentation were noted. The main result of this experiment is that the antiscorbutic vitamin system is shown to be most stable in the region of the natural p_H of lemon juice, *i.e.* p_H 1·8–2·2.

To test whether any precipitation of the vitamin took place in the more acid solutions of juice (p_H 1·4 and above), the residues of these juices were made up with distilled water to a total volume which was $\frac{2}{3}$ of the original juice preserved. Immediately before administering to test animals, the samples were shaken up well to ensure perfect mixing. 10 cc. of each sample were taken and brought to the normal p_H of lemon juice, the final volume being in each case 20 cc. Daily doses of 2·0 cc., 4·0 cc. and 6·0 cc., corresponding to 1·5, 3·0, 4·5 cc. of the original juice were given, the usual technique being followed. Precautions were taken to ensure that representative samples were given to the test animals, by mixing between each dosage (Table VII).

Table VII. *Residues of juice from experiments in Table VI.*

Sample of juice	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions
Residue from juice + HCl p_H 0·6	429	♀	1·5	19	All these guinea-pigs were found to show severe extensive haemorrhages and beading of costo-chondral junctions. Acute scurvy
	436	♂	3·0	19	
	424	♂	4·5	22	
Residue from juice + HCl p_H 1·0	427	♀	1·5	19	
	435	♂	3·0	22	
	423	♀	4·5	19	
Residue from juice + HCl p_H 1·4	434	♂	1·5	22	
	437	♂	3·0	22	
	428	♀	4·5	19	

From this it will be seen that precipitation of the antiscorbutic vitamin does not take place in acid solution but that complete destruction of this factor occurs.

III. The effect of other preservatives on the antiscorbutic potency of lemon juice.

Formic acid and sodium benzoate.

Two samples of lemon juice, preserved with 0·05 % sodium benzoate and 0·25 % formic acid, respectively, were obtained from Sicily. On arrival in the laboratory both of these samples had fermented slightly as was evidenced by the escape of gas when the samples were opened. It was apparent that the preservation against gross fermentation exhibited by these two acids is inferior to that by similar amounts of sulphur dioxide in the form of potassium meta-bisulphite. Moreover, the palatability of the juices preserved by sulphur dioxide was superior to that of those preserved by sodium benzoate and formic acid.

From the time of pressing until the commencement of the animal feeding tests about a month elapsed. Doses ranging from 1·0 cc. to 3·0 cc. of each of the two samples were administered to the test guinea-pigs (Table VIII).

Table VIII. *Preservative action of sodium benzoate and of formic acid.*

Sample	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions
Lemon juice + 0·05 % sodium benzoate	332	♂	1·0	50	Slight femoral haemorrhages, sub-maxillary and jaw more severe—scurvy
	334	♀	1·5	50	Severe haemorrhages—acute scurvy
	344	♂	2·0	74	Extensive haemorrhages—acute scurvy
	309	♀	2·5	88	Found dead: haemorrhages and emaciated condition—scurvy
	321	♀	3·0	74	Extensive haemorrhages—acute scurvy
Lemon juice + 0·25 % formic acid	307	♂	1·0	43	Extensive haemorrhages—acute scurvy
	328	♂	1·5	57	Extensive haemorrhages—acute scurvy
	326	♂	2·0	60	Extensive haemorrhages—acute scurvy
	317	♂	2·5	86	Haemorrhages—slight scurvy
	314	♂	3·0	96	Haemorrhages—incipient scurvy

It will be seen that 0·05 % benzoic acid and 0·25 % formic acid are as inefficient as 0·04 % metabisulphite as preservatives of the antiscorbutic vitamin. Furthermore, they keep the juice neither palatable nor of good colour.

Essential oil of cloves.

Essential oils, such as oil of cloves, which have mild antiseptic properties, have been used for the preservation of wine and other substances which suffer deterioration from fermentation. A sample of juice pressed in the laboratory and preserved with 0·1 % oil of cloves was kept for 4–5 weeks at room temperature. No signs of mould growth or fermentation could be detected and the sample of juice was palatable and of good colour (Table IX).

Table IX. *Preservative action of oil of cloves.*

Sample	Animal	Sex	Dose (cc.)	Duration of exp. (days)	*Post-mortem* and general conclusions
Juice + 0·1 % oil of cloves	419	♀	1·5	23	Extensive haemorrhages and beading of rib junctions —acute scurvy
	412	♂	2·0	23	Extensive haemorrhages and beading of rib junctions —acute scurvy
	421	♀	2·5	23	Femoral haemorrhages: slight beading of rib junctions—scurvy
	422	♀	3·0	23	Femoral haemorrhages: slight beading of rib junctions—scurvy

It will be seen that a daily dose of 3·0 cc. of lemon juice preserved with 0·1 % oil of cloves is insufficient to protect against scurvy over 23 days. In fact, it appears that the antiscorbutic potency is lost as completely as with potassium metabisulphite

Glucose and sucrose.

Since sugars and syrups are employed in the preparation of concentrated and dried fruit juice, it appeared advisable to test whether sucrose and glucose acted as preservatives for vitamin C in fresh lemon juice.

Small samples (400 cc.) were placed aside in March 1928, preserved with 30 % glucose, 10 %, 20 %, 30 % and 50 % sucrose.

In February 1929, *i.e.* 11 months after storage, the appearance of the juices was noted (see Table X).

Table X.

Sample	Appearance	Remarks
Juice + 30 % glucose	Slight mould	All the samples smelt as if slightly fermented and there was a slight gas evolution
Juice + 10 % sucrose	Slight mould	
Juice + 20 % sucrose	Slight mould	
Juice + 30 % sucrose	No mould	
Juice + 50 % sucrose	No mould	

Daily doses of 1·5 cc. and 3·0 cc. of three of these samples, lemon juices preserved with 30 % glucose, 10 %, and 30 % sucrose respectively were fed to experimental guinea-pigs, the usual technique being followed (Table XI).

Table XI. *Preservative action of glucose and sucrose respectively.*

Juice	Animal	Sex	Dose (cc.)	Duration of expt. (days)	*Post-mortem* and general conclusions
Lemon juice + 10 % sucrose	445	♀	1·5	24	Scurvy. Femoral and costochondral haemorrhages and beading
	447	♂	3·0	21	Acute scurvy. Severe extensive haemorrhages
Lemon juice + 30 % sucrose	448	♀	1·5	19	Acute scurvy. Femoral haemorrhages and beading of costochondral junctions
	449	♀	3·0	19	Acute scurvy. Femoral haemorrhages and beading of costochondral junctions
Lemon juice + 30 % glucose	444	♀	1·5	26	Very small haemorrhages, femur and costochondral junctions: no beading or other scurvy symptoms. Emaciated condition
	446	♀	3·0	48	No characteristic scurvy, haemorrhages or beading. Very emaciated condition

Judging from the condition of the samples of lemon juice, the various concentrations of sugar were sufficient to inhibit to a great extent the growth of bacteria and moulds. This is in virtue of the osmotic effect. When the anti-scorbutic potency of the juices is compared an interesting difference between the behaviour of the two sugars, sucrose and glucose, is noted.

The juices preserved with sucrose had lost all their antiscorbutic potency, the test animals having developed acute scurvy within 3 weeks. Unfortunately the test of the juice containing glucose could not be carried to a conclusive point as the animals became emaciated and suffered from digestive troubles, probably as the result of the high concentration of glucose. Whilst it cannot be said definitely that glucose is a good preservative for the antiscorbutic vitamin, it is obvious that there is an essential difference between the reducing sugar, glucose, and the non-reducing sugar, sucrose, in connection with

Table XII. *Summary of results.*

Preservative	Minimum protective dose	General condition of juice
0·02 % potassium metabisulphite	> 3·5 cc. after 2½ months' preservation	Clear yellow colour: heavy growth of moulds on surface
0·04 % potassium metabisulphite	> 3·0 cc. after 1 month's preservation	Clear: no fermentation
Juice kept in sulphured cask	2·0–2·5 cc. after 1 month's preservation	Cloudy and fermented
0·07 % lemon rind oil	2·5 cc. after 2½ months' preservation	Slightly cloudy: slight growth of moulds
0·275 % lemon rind oil	3·0–3·5 cc. after 2½ months' preservation	Slightly cloudy: no moulds
0·5 % lemon rind oil	3·0–3·5 cc. after 2½ months' preservation	Clear: no moulds
1·1 % lemon rind oil	> 3·5 cc. after 2½ months' preservation	Clear: no moulds
0·07 % lemon rind oil	2·0–2·5 cc. after 1 month's preservation	Cloudy: some fermentation
0·275 % lemon rind oil	2·5 cc. after 1 month's preservation	Cloudy: some fermentation
Hydrochloric acid (p_H 1·6)	1·5 cc. after 1 month's preservation	Yellow, cloudy and fermented
Hydrochloric acid (p_H 1·6)	1·5 cc. after 1½ months' preservation	Badly fermented
Hydrochloric acid (p_H 0·6)	> 3·0 cc. after 7–8 months' preservation	No mould growth or fermentation. The juice turned brown, possibly due to hydrolysis of protein in the juice
Hydrochloric acid (p_H 1·0)		
Hydrochloric acid (p_H 1·4)		
Hydrochloric acid (p_H 1·8)	1·5–2·0 cc. after 7–8 months' preservation	Slight growth of mould and darkening of juice
Unpreserved lemon juice (p_H 2·2)	1·5–2·0 cc. after 7–8 months' preservation	Heavy mould growth and alcoholic fermentation
Sodium hydroxide (p_H 3·0)	3·0 cc. after 7–8 months' preservation	Heavy mould growth and alcoholic fermentation
0·05 % sodium benzoate	> 3·0 cc. after 1 month's preservation	Brown, cloudy and fermented
0·25 % formic acid	> 3·0 cc. after 1 month's preservation	Brown, cloudy and fermented
0·1 % oil of cloves	> 3·0 cc. after 1 month's preservation	Palatable and good colour; no fermentation
10 % sucrose	> 3·0 cc. after 11 months' preservation	Slight mould growth and fermentation
30 % sucrose	> 3·0 cc. after 11 months' preservation	No mould growth; slight fermentation
30 % glucose	< 3·0 cc. after 11 months' preservation	Slight mould growth and fermentation

vitamin C. The animal receiving 1·5 cc. of the juice containing glucose showed no definite signs of scurvy after 26 days, while that receiving 3·0 cc. showed no symptoms after 48 days. It may be gathered therefore that this sample of juice retained a fair amount of its antiscorbutic potency, but no definite conclusions can be drawn.

Discussion.

Table XII summarises the results on the effect of various preservatives on lemon juice given in the present paper.

These results are very striking. They indicate that those substances which exert the strongest preservative effect against gross fermentation possess the greatest destructive action on the antiscorbutic vitamin system. The juice which contained no added preservative retained its antiscorbutic potency to a great extent. The results with acid are particularly significant, since they indicate that the antiscorbutic vitamin is extremely unstable under conditions which are detrimental to the development of moulds and bacteria.

Summary.

1. Potassium metabisulphite, whilst being the best preservative for lemon juice against fermentation, has a definite destructive action on the antiscorbutic vitamin at laboratory temperature (15°–18°). This destruction proceeds with a measurable velocity.

2. Lemon rind oil, whilst not preserving lemon juice satisfactorily against fermentation at ordinary temperature (15°–18°), is less destructive towards vitamin C than potassium metabisulphite. A concentration of 0·07 % rind oil preserves the vitamin potency slightly better than 0·275 % rind oil, indicating that rind oil has a slight destructive action on the vitamin system.

3. The optimal zone of stability of the antiscorbutic vitamin in lemon juice at ordinary temperature lies between p_H 1·6–2·2, that is, in the neighbourhood of the natural acidity of lemon juice.

4. Lemon juice adjusted with HCl to p_H 1·6 preserves its antiscorbutic activity for 14 months at ordinary temperatures.

5. Sodium benzoate and formic acid exert a destructive action on vitamin C.

6. Oil of cloves exerts a destructive action on the antiscorbutic vitamin comparable with that of 0·04 % potassium metabisulphite.

7. Sucrose fails to preserve vitamin C in lemon juice. Indications are given that glucose may exert a preservative action on the vitamin.

8. It is pointed out that the antiscorbutic vitamin system is particularly unstable under conditions detrimental to the development of moulds and bacteria.

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