XX. THE ANTI-SCORBUTIC PROPERTIES OF CONCENTRATED FRUIT JUICES. PART III.

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IN a previous paper [1919] a method was described by which orange juice could be reduced, by evaporation at low temperatures, to a crisp, dry solid, retaining in a considerable degree the anti-scorbutic properties of the raw juice. This residue, which was very hygroscopic, was stored in a desiccator over sulphuric acid, at room temperature. Tests on guinea-pigs were carried out when the material was from four to seven months old and showed that a daily ration of 0.5 g., equivalent to about 4.5 cc. of the raw juice, afforded complete protection from scurvy, but the minimum daily amount sufficient to afford such protection was not determined. A quantity of the dried juice was set aside for further tests after a longer interval of time, and these tests have now been carried out. The material formed part of the original quantity prepared in June 1917 and since then had been stored in a desiccator at room temperature. The tests were carried out during April-June 1919 so that the average age of the dried juice was nearly two years. Unfortunately the amount available did not permit of an extended series of tests in order to determine the minimum dose and it was thought advisable to repeat the somewhat high daily ration (0.5 g.) used in the previous tests rather than risk more inconclusive results by feeding single animals on varying smaller amounts. The results of these tests are shown below. In these and other experiments described in this paper the animals employed were guinea-pigs and the basal diet consisted of oats and bran *ad lib.* with 60 cc. daily of milk autoclaved at 120° for one hour.

Table I.

No. of animal	Diet	Initial weight of animal (g.)	Condition of animal during course of experiment	Length of experiment (days)	Final weight of animal (g.)	Result
136	Basal + 0.5 g. dried orange juice daily from the 13th day	326	Weight increased steadily throughout the experiment (maximum weight 519 g. on 75th day). No symptoms of scurvy were observed	77	503	Animal in good health; killed on 77th day; no signs of scurvy
137	Basal + 0.5 g. dried orange juice daily from the 13th day	307	Weight increased steadily throughout the experiment (maximum weight 436 g. on 75th day). No symptoms of scurvy were observed	77	422	Animal in good health; killed on 77th day; no signs of scurvy

It will be seen that the daily ration of dried orange juice was not given until the 13th day of the experiment. This was on account of the limited amount of the material available, but the advisability of the procedure is questionable. The animal's reserve stock of the anti-scorbutic accessory factor must have been very much reduced and this may perhaps account for the somewhat low rate of growth shown by guinea-pig 137.

Microscopical examinations of the ribs of both animals were very kindly carried out by Miss Tozer to whom we are indebted for the following reports:

Guinea-pig 136:—"The two ribs examined are practically normal, a slight shortening of the trabeculae being the only abnormality."

Guinea-pig 137:—"The two ribs examined are normal."

In these experiments, therefore, complete protection from scurvy was afforded by a daily ration of 0.5 g. of dried orange juice, after this had been kept during nearly two years in a dry atmosphere at room temperature. This quantity was equivalent to about 4.5 cc. of the raw juice.

From the work of Chick and Rhodes [1918] and Davey [quoted by Delf, 1920] it would appear that the minimum daily ration of raw orange juice sufficient to protect a guinea-pig completely from scurvy is about 1.5 cc., though it seems probable that this figure may vary to some extent with the character and age of the fruit. Hence the experiments described above do not exclude the possibility of considerable loss of the anti-scorbutic factor either during the process of drying or during storage. It can only be stated that if such loss took place it probably did not exceed 66 % of the amount originally present in the raw juice. It seemed advisable, therefore, to repeat these experiments on a larger scale in order to decide whether any loss of the anti-scorbutic factor actually occurs. Accordingly a considerable quantity of dried orange juice was prepared during November and December 1919. The oranges were fine new season's fruit and yielded a high proportion of juice which was, however, very tart. It was clarified by centrifuging and then passing through filter paper in a Buchner funnel. Five litres of juice containing 10.0 % of total solids were evaporated in quantities of 500-600 cc. at a time, the temperature of the bath being maintained at 50°-52° while the receiver was surrounded by ice. About four hours were required to evaporate this quantity of juice to a thick syrup, the final drying of which was completed by the method previously described. One portion of the dry product has been stored in a screw-top glass bottle, and kept at a uniform temperature of 26°. It is intended to test this portion after a lapse of one year. With another portion animal experiments were at once commenced, two guinea-pigs receiving 0.15 g., two receiving 0.3 g. and two receiving 0.45 g. daily, equivalent to 1.5, 3.0 and 4.5 cc. of raw juice respectively. On account of the tartness of the juice a little cane sugar was added to the ration so that the animals should take it readily. As will be seen from the tables below, all six animals were satisfactorily protected from scurvy during the 85 days that the experiment lasted and we may therefore conclude that no appreciable loss of the anti-

scorbutic factor occurs when orange juice is evaporated to dryness under the given conditions.

Table II.

No. of animal	Diet	Initial weight of animal (g.)	Condition of animal during course of experiment	Length of experiment (days)	Final weight of animal (g.)	Result
156	Basal + 0.15 g. dried orange juice daily from 1st day	283	Weight increased throughout experiment. Maximum weight 550 g. on the 83rd day. No symptoms of scurvy were observed	85	538	Animal in good health; killed on the 85th day; no signs of scurvy
157	Basal + 0.15 g. dried orange juice daily from 1st day	282	Weight increased throughout experiment. Maximum weight 490 g. No symptoms of scurvy were observed	85	490	Animal in good health; killed on the 85th day; no signs of scurvy
158	Basal + 0.3 g. dried orange juice daily from 1st day	283	Weight increased throughout experiment. Maximum weight 573 g. on the 75th day. No symptoms of scurvy were observed	85	555	Animal in good health; killed on the 85th day; no signs of scurvy
159	Basal + 0.3 g. dried orange juice daily from 1st day	332	Weight increased throughout experiment. Maximum weight 627 g. on the 75th day. No symptoms of scurvy were observed	85	613	Animal killed on 85th day. Post mortem examination revealed acute congestion of the left lung but there were no signs of scurvy
160	Basal + 0.45 g. dried orange juice daily from 1st day	283	Weight increased throughout experiment. Maximum weight 557 g. on the 75th day. No symptoms of scurvy were observed	85	525	Animal in good health; killed on the 85th day; no signs of scurvy
161	Basal + 0.45 g. dried orange juice daily from 1st day	300	Weight increased throughout experiment. Maximum weight 506 g. on the 82nd day. No symptoms of scurvy were observed	85	505	Animal in good health; killed on the 85th day; no signs of scurvy

[NOTE:—On the 75th day the animals were moved into another building. As a result of this change five of the animals suffered a temporary fall in weight from which some had not completely recovered when killed on the 85th day.]

That considerable loss of anti-scorbutic potency may occur when other methods of evaporation are employed is shown by the experiments of Givens and McClugage [1919] in which orange juice in shallow dishes was dried by a current of air heated to 55°–60°, the duration of drying varying from 36 to 63 hours. A daily ration of the dried product equivalent to 12.5 cc. of the raw juice was required in order that guinea-pigs might be completely protected from scurvy

whereas on the equivalent of 6.25 cc. the animals developed scurvy in less than 20 days. The loss by this method must therefore have amounted to more than 76 %.

It had been our intention to extend this investigation to the various industrial processes for the rapid evaporation of liquids, particularly those used in the preparation of milk powders, in the hope that one of these processes or some practicable modification might yield from orange juice a product of high anti-scorbutic potency, that could be stored for considerable periods under varying conditions of climate, without serious loss of the accessory factor. Preliminary experiments had been commenced with the co-operation of the Kestner Evaporator Co. Ltd., to whose managing director Mr J. A. Reavell and chemist Mr Nicol we are greatly indebted for much valuable assistance. In July 1919 Givens and McClugage published an account of some successful experiments with dried orange juice prepared by the Merrell-Soule patented process for making milk-powder. The orange juice, mixed with corn-syrup, was forced as a fine spray into a chamber where it met a current of air heated to 75°–80°. The drying was almost instantaneous and the product, when tested on guinea-pigs, was found to afford complete protection from scurvy. The daily dose given to the animals was 1 g. equivalent to 3 cc. of raw juice, which was taken, from the earlier experiments of Chick and Rhodes [1918], as the minimum daily dose of fresh orange juice sufficient to afford complete protection. Givens and McClugage state that they intend to report on the activity of this product after a long period of storage and it is to be hoped that smaller doses will also be tested.

In view of the satisfactory results quoted above it appeared to us unnecessary to continue our attempts, for there would seem to be little doubt that a highly active dried orange juice can be readily prepared on a commercial scale, and from our own experiments recorded in this paper it seems very probable that such a product will retain its potency after prolonged storage under suitable conditions. Orange juice in fact would appear to be particularly suited for the preparation of a dried anti-scorbutic, as it has been shown by Delf [1920] to lose very little of its potency, even when heated at 100° for an hour. The peculiar value of such a substance would lie in its adaptability for infant feeding and for the use of expeditions of long duration, where fresh fruit and vegetables are unobtainable and transport must be reduced to a minimum. The investigation of the effects of storage at tropical temperatures is therefore advisable.

A brief account of the preliminary experiments on large scale evaporation mentioned above may here be given.

(A) The juice of 360 oranges (18.5 litres) was strained through muslin and concentrated in the "Kestner Evaporator," under 25 inches of vacuum and a steam pressure of 10–15 lbs. The time taken for the passage of any portion of the juice through the evaporator was less than one minute and the temperature of the escaping liquid was below 60° (usually below 50°). Owing

to the juice being insufficiently clarified its passage through the inlet valve was somewhat erratic and the consequent evaporation less than the maximum obtainable under the stated conditions of temperature and pressure. It was consequently necessary to pass the juice three times through the evaporator in order to obtain the desired degree of concentration. The product was a viscous syrup containing 68 % of total solids as against 11.6 % in the original juice. A portion of this syrup was tested on guinea-pigs (see Table III, A), and during the course of the experiments was kept in a loosely-corked bottle at room temperature. Two guinea-pigs, each receiving a daily ration of 0.14 g., equivalent to 0.8 cc. of raw juice, developed symptoms of scurvy in 26 days and died in 42 and 39 days respectively. Two guinea-pigs received a daily ration of 0.27 g., equivalent to 1.6 cc. of raw juice. Unfortunately the condition of both animals was unsatisfactory from the first and there was no appreciable gain in weight even during the initial 14 days. In one animal scurvy symptoms were observed after 36 days and the weight commenced to fall slowly. No such symptoms were observed in the other animal though its general condition was not good and its weight declined after the 52nd day. Both animals were killed on the 73rd day and signs of scurvy were found in each case. Two animals received a daily ration of 0.67 g., equivalent to 4.0 cc. of raw juice; one of these was completely protected from scurvy during 93 days but in the other scurvy symptoms were observed after 77 days. From these results it might appear that a loss of anti-scorbutic potency, amounting to more than 63 %, had occurred during concentration of the orange juice. It is very possible, however, that the actual loss due to the process of concentration was considerably less than this, but that further loss occurred during storage in the course of the animal tests, and some evidence in favour of this view can be derived from a comparison of the results obtained with the different doses and with the corresponding amounts of the dried product after further treatment (see below).

(B) The bulk of the concentrated syrup described above was submitted to further concentration in a small vacuum pan fitted with a mechanical stirrer and scraper. A vacuum of 24–25 inches was maintained but the evaporation was very slow and it was found necessary to raise the temperature of the outer bath to 80° for 2½ hours. Even then the result was unsatisfactory, the product being a viscous mass, very difficult to remove from the pan. On cooling it set to a dark coloured, toffee-like solid containing 7.5 % of water.

The final drying of orange juice by this method was obviously impracticable but the product still retained its anti-scorbutic potency in greater degree than might have been expected after such drastic treatment (see Table III, B). Two guinea-pigs, each receiving 0.1 g. daily, equivalent to 0.8 cc. of raw juice, developed symptoms of scurvy in 26 and 31 days and died in 39 and 44 days respectively. A daily ration of 0.2 g. also afforded only partial protection, but two guinea-pigs each receiving 0.5 g. daily, equivalent to 4.0 cc. of

Table III.

A (Concentrated orange juice : total solids = 68%)							B (Dried orange juice : total solids = 92.5%)						
No. of animal	Daily ration of "A"	Initial weight of animal (g.)	Condition of animal during course of experiment	Length of experiment (days)	Final weight of animal (g.)	Result	No. of animal	Daily ration of "B"	Initial weight of animal (g.)	Condition of animal during course of experiment	Length of experiment (days)	Final weight of animal (g.)	Result
138	0-14 g. ¹ (0-8 cc.)	364	Maximum weight 368 g. on the 12th day. Scurvy symptoms observed on the 26th day	42	217	Death from scurvy on the 42nd day	144	0-1 g. (0-8 cc.)	312	Maximum weight 356 g. on the 12th day. Scurvy symptoms observed on the 26th day	39	207	Death from scurvy on the 39th day
139	0-14 g. (0-8 cc.)	332	Maximum weight 367 g. on the 19th day. Scurvy symptoms observed on the 26th day	39	232	Death from scurvy on the 39th day	145	0-1 g. (0-8 cc.)	312	Maximum weight 323 g. on the 12th day. Scurvy symptoms observed on the 31st day	44	193	Death from scurvy on the 44th day
140	0-27 g. (1-6 cc.)	399	Maximum weight 420 g. on the 19th day. Scurvy symptoms observed on the 36th day	73	301	Animal killed on the 73rd day: severe scurvy	146	0-2 g. (1-6 cc.)	339	Maximum weight 375 g. on the 19th day. Scurvy symptoms observed on the 33rd day	52	233	Death from scurvy on the 52nd day
141	0-27 g. (1-6 cc.)	318	Weight remained constant during 52 days and then fell slowly. No symptoms of scurvy observed but general condition bad	73	241	Animal killed on the 73rd day: signs of mild scurvy	147	0-2 g. (1-6 cc.)	287	Maximum weight 338 g. on the 24th day. Scurvy symptoms observed on the 47th day	73	266	Animal killed on the 73rd day: severe scurvy
142	0-67 g. (4-0 cc.)	349	Maximum weight 442 g. on the 80th day. No symptoms of scurvy observed	93	440	Animal killed on the 93rd day: no signs of scurvy	148	0-5 g. (4-0 cc.)	317	Maximum weight 423 g. on the 50th day. No symptoms of scurvy observed	93	405	Animal killed on the 93rd day: no signs of scurvy
143	0-67 g. (4-0 cc.)	285	Maximum weight 448 g. on the 61st day. Scurvy symptoms observed on the 77th day	93	341	Animal killed on the 93rd day: scurvy not very severe	149	0-5 g. (4-0 cc.)	290	Maximum weight 431 g. on the 78th day. No symptoms of scurvy observed but the animal did not appear in good health. From the 94th day it was given a highly antiscorbutic diet but its condition did not improve, and it was killed on the 115th day, its weight being 402 g.	93	425	Animal killed on the 115th day. No signs of scurvy were found but there was acute congestion of both lungs

¹ The figures in brackets give the equivalent amount of raw orange juice.

raw juice, did not show any symptoms of scurvy during 93 days, though their general condition was not entirely satisfactory and the normal rate of growth was not maintained throughout the whole period.

SUMMARY.

Orange juice can be reduced by evaporation under suitable conditions to a dry residue without suffering appreciable loss of the anti-scorbutic accessory factor, and this residue still retains a considerable degree of potency after storage during two years in a dry atmosphere at ordinary temperatures.

The preparation on a commercial scale of such a dried orange juice appears to be quite practicable, and should prove of considerable value where an anti-scorbutic material is required in a highly concentrated and stable form.

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