

A NEW METHOD FOR THE ESTIMATION OF VITAMIN-C

VITAMIN-C is the only vitamin for which reliable and simple chemical methods are found. The one generally employed is Tillman's 2:6-dichlorophenol indophenol dye titration method. But this method suffers from the disadvantage of lack of precision in the end-point with old solutions of the dye, while with fresh solutions the experimental procedure becomes tedious. Several alternative methods also are to be found, and of these excepting the method of Roe & his collaborators¹⁻³ in which the red colour produced by dehydro-ascorbic acid with 2:4-dinitro-phenylhydrazine is measured, all are based on the reducing property of vitamin-C on several reagents, e.g., methylene blue,⁴ phospho-18-tungstic acid,⁵ silicomolybdic acid⁶ arsenotungstic—and arsenotungstomolybdic acids⁷ and ferridipyridyl sulphate.⁸ We tried Folin's phosphomolybdic acid reagent (containing tungstic acid) for sugar and found that it gives blue colour with vitamin-C at room temperature only very slowly, taking many hours to reach the maximum. However, the colour develops rapidly at 100° C., but under these conditions fructose also produces the blue colour.

Next our choice fell on the phosphomolybdic acid reagent of Polis and Sortwell⁹. We found that this reagent gives even in the cold (30° C) an intense blue colour with such small concentrations of the vitamin as M/1000. The colour is found to be quite stable for several hours.

A number of substances (citrate, oxalate, malonate, malate, etc.) and sugars present in fruits were tested and none is found to give any colour in the cold. On heating to 100° C. few sugars gave colour, fructose yielding comparatively more colour. Cysteine and glutathione (GSH) gave no colour in the cold even with M/100 solutions. On keeping, however, a faint greenish blue colour began to develop slowly. These thiol compounds produced greenish blue colours in high concentrations, e.g. M/10, but the colours took several hours to reach the maximum intensities.

The method we have worked out, for the present for pure aqueous solutions of ascorbic acid, is as follows.

5 ml. of vit-C (M/1000 to M/100) in 10 per cent. acetic acid are treated with 0.2 ml.

of the phosphomolybdic acid reagent and shaken well. A blue colour rapidly develops and reaches its maximum intensity in about 5 mins. The colour is found to be quite stable and proportional to the concentration of vit-C. The colour can be estimated colorimetrically, preferably on a photo-electric colorimeter. A further advantage of our method lies in the fact that the blue reduction compound can be estimated by titration against M/100 potassium permanganate from a micro-burette, till the blue colour disappears and there remains only a yellow colour due to the reagent. From the titre the vit-C content can be calculated.

Further work on the application of the method for foods and biological fluids is in progress.

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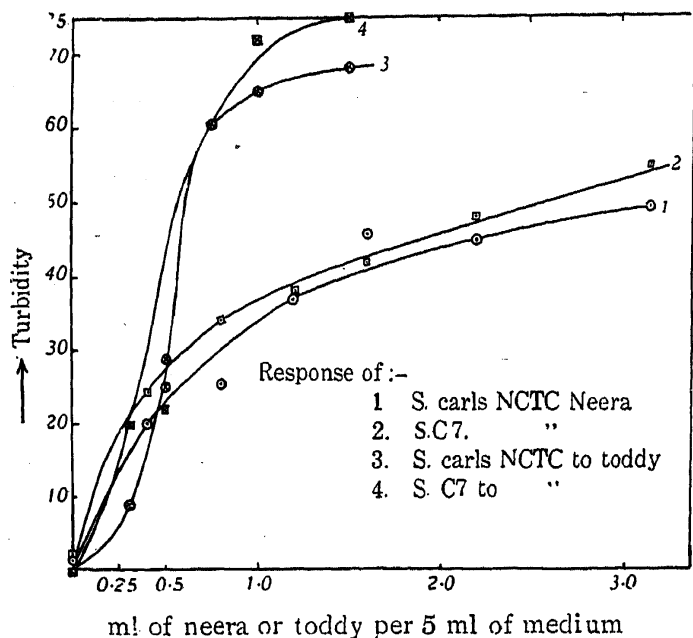
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OVERALL GROWTH-PROMOTING POTENCY OF NEERA (UNFERMENTED JUICE) AND TODDY (FERMENTED BEER) FROM PHOENIX SYLVESTRIS

POWERFUL growth-promoting and therapeutic properties are popularly attributed to Neera or the sweet unfermented juice of the palmyra palm, *Phoenix sylvestris*. The problem of Neera—its preservation, nutritional value and its economic disposal—has, at the moment, attained great topical significance, and as part of a comprehensive scheme of investigation, the overall growth-promoting potency of the unfermented and fermented forms of Neera

has been determined by a microbiological method described earlier.¹

Two cultures of yeasts, SC 9, NCTC 3006 and *S. carlsbergensis* NCTC 3056, whose vitamin requirements were previously determined, have been employed for all the assays and the results are graphically represented below:—



The results show that fresh and unfermented neera possesses a low overall growth-promoting potency whereas the fermented counterpart exhibits a comparatively far higher potency. As could be gathered from the curves, the potency is doubled indicating a two-fold enrichment of the growth factors during the fermentation. This is not an unexpected result in view of the well-established fact that micro-organisms synthesise and secrete vitamins during their growth. This finding would point to the perhaps provocative conclusion that if the B-complex requirements are to be met from this source, fermented toddy should be prescribed even against the policy of prohibition—a conclusion which is in conformity with the observations of Professor J. B. S. Haldane who, under the arresting caption of "Tragedy of Nauru," records that the infantile mortality among the Pacific islanders rose to 50 per cent. within six months of the enforcement of prohibition on the island. He adds "It was found that the children at the breast were dying of beri-beri, a disease due to the deficiency of thiamin. This substance is nearly absent from the rather monotonous diet of the mothers, but is present in large quantities in the yeast from which the toddy

is made. The Medical Officer of Health discovered this fact and (doubtless after an appropriate delay) toddy was allowed again. The infant mortality fell to 7 per cent.

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THE EFFECT OF COCOANUT WATER ON THE GROWTH OF IMMATURE EMBRYOS OF CORN (*MAIZE*)

TUKEY (1933 and 1934) suggested that the very young sweet cherry embryos required for their growth certain heat stable factors found in yeast and fibrin digest in addition to sugar. Van Overbeek and his associates (1941) in their work on *Datura* embryo found that embryos that did not grow in Tukey's medium containing mineral elements, did grow when to this physiologically active substances were added. Coconut water in addition, was found to help better growth in very young embryos.

In an independent experiment designed to find out the period of viability for three-week old embryos excised from corn kernels that were cold stored (Uttaman, 1949) the effect of coconut water on the growth of the embryos was noted. The embryos that were daily dissected out of a number of kernels under aseptic conditions were placed in Tukey's general purpose medium and the effect of coconut water on their germination and growth daily watched. Water from mature nuts was cold preserved at freezing point and used as and when required. They were tried in two sets. To one was added coconut water daily at the rate of 3 drops for each embryo and to the other no coconut water was added. Growth measurements were made for 4 embryos under each set, each embryo being measured for two successive days beginning from the 3rd day of placing in the medium and finally on the 5th day. The results are recorded in the table below.

It will be noted from the table that coconut water has a decisive depressing effect