Information on Vitamin C

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Once we found that when we determined vitamin C in some vegetable especially in some coloured sample, we could accomplish the decolourization with a bit of fuller's earth, keeping vitamin C stable.

But the indophenol method has a very weak point that the indophenol aqueous solution is very unstable, that is to say, if the solution is kept for more than five days (after it is compounded), it makes the experimental results obscure, as it turns the last stage of titration brown.

To improve such a weak point we experimented on solvents of 2 : 6-dichlorphenolindophenol.

Standing on the data of our experiment, we advocate the following : dissolve 2 : 6-dichlorphenolindophenol in nor- or iso-butylalcohol, and at the time of titration, use it after diluting it with distilled water or 94% ethanol. And when indophenol water saturated with butanol solution or indophenol-butanol-ethanol solution is kept in a dark and cold place, it becomes much more stable and more durable than the normal aqueous solution of indophenol, and moreover, this solution is not discolored by acids, and thus the only great defect of the indophenol method can be perfectly remedied.

Now, we would like to discuss one of the most representative sources of vitamin C in the Orient, the green tea.

Determing vitamin C in the various sorts of tea, we found that the vitamin C content in Sencha (Green tea of the superior quality) and Bancha (Green tea of the inferior quality) is greater than in Gyokuro (Green tea of the special and superior quality), Matcha (Powdered tea of the superior quality) and black tea.

We sufficiently explained the cause of this difference basing on our experiments on the relation between the vitamin C content and the sunlight, and on its stability to heat.

Lack of vitamin C in the human body causes some derangement of the circulatory system, and the blood congests in the capillary vessels to break them and flows.

When one is short of fresh vegetable foods, like some seafaring men, he will first turn pale, and then purple spots will appear on his skin due to the rupture of the blood vessels, and he will feel a pain in his hip, and his gums will become purple, and he will have difficulty in breathing and according to circumstances he may die after all, though these are not likely to happen to grown-ups who are taking ordinary diet.

Scurvy arises from lack of vitamin C. Babies being artificially fed or too particular about their choice for food are often taken ill with infantile scurvy, and especially sucklings and women in pregnancy or those nursing infants must take vitamin C together with other vitamins.

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Let us look back over the years of researches in vitamin C. As H. R. Rosenberg, Sc. D. said in his "Chemistry and Physiology of the Vitamins", scurvy, the typical syndrome of a vitamin C deficiency, has been known for many centuries and occurred epidemically during times of war, voyages, famines, etc. Fresh vegetables have been known to provide a potent remedy for over three hundred years.

1903-1913 : Bolle, Bartenstein and later Holst and Frölich observed that guinea pigs could acquire scurvy just as men could, and that the scurvy syndrome is a condition caused by an avitaminosis.

1918-1925 : Zilva attempted the concentration of the antiscorbutic substance from lemon, obtained almost pure ascorbic acid.

1927 : On the basis of the observation that vitamin C solutions immediately after mild oxidation retained their antiscorbutic properties, Zilva[1] concluded that the antiscorbutic compound and the "reducing factor" were closely related although not necessarily identical.

1928 : Szent-Györgyi[2] isolated from adrenal glands, from oranges and from cabbage a strongly reducing compound, the "hexuronic acid".

1932 : The identity of vitamin C with Szent-Györgyi's hexuronic acid and with Zilva's "reducing factor" was discovered by various groups of workers, namely by Svirbely and Szent-Györgyi,[3] by Waugh and King,[4] and by Tillmans[5] who deduced that vitamin C can be reversibly oxidized and reduced without loss of antiscorbutic efficacy.

1933 : The constitution of vitamin C was established by the combined work of Haworth, Hirst and co-workers[6] and of Micheel and Kraft[7]. Reichstein[8] and Haworth[9] announced the first successful synthesis of ascorbic acid.

Vitamin C is widely dirstributed over the animal and plant kingdoms. It is present in all living plant cells and augmented amounts are found in all actively growing parts of higher plants.

Hitherto, however, vitamin C in various plants has not widely studied, and as to its physiological action and the significance of its existence in plants, many questions remain unsolved.

We shall first explain briefly our method of the titrimetry of vitamin C and then introduce to you the experimental results obtained by our improved method, and illustrate several properties of vitamin C contained in tea which is most intimately related to the vitamin.

Once we found that when we determined vitamin C in some vegetables especially in some coloured samples, we could accomplish the decolourization keeping vitamin C stable, by first extracting the sample with acetic acid, then precipitating protein in extractive matters, by metaphosphoric acid, and finally decolourizing the matters with a bit of fuller's earth.

And we recommended that to point out exactly the limit of the discolouration in the titrimetric determination of vitamin C with 2:6-dichlorphenolindophenol, aqueous solution one should add a few drops of ether (butanol) in the test-tube, then the indophenol which remained coloured, penetrates the layer of ether (butanol) immediately.

Thus, one can know whether titration is complete or not. But the indophenol method has a very weak point that the indophenol aqueous solution is very unstable, that is to say, if the solution is kept for more than five days (after it is compounded) it makes the experimental results obscure, as it turns the last stage of titration brown.

To improve such a weak point, we experimented on solvents of 2:6-dichlorphenolindophenol.

Standing on the data of our experiments, we advocate the following methed: saturate nor- or iso-butylalcohol with 2:6-dichlorphenolindophenol very carefully, and after keeping the saturated solution for a whole night, filter it and prepare the storable mother liquor.

Though indophenol of a certain quality is very hard to dissolve, try, for example, to dissolve 250 mg of indophenol in butylalcohol to the utmost, and then filter it, and fill it up into 100 ml messflasks, then you can prepare the storable mother liquor (I. B.).

Unlike the mere indophenol aqueous solution, the indophenol butanol solution (I. B.), is stable for several months, and it becomes stabler when it is put into a coloured bottle and stored in an ice-box.

At the time of titration, prepare indophenol water saturated butanol solution (I. B. W.), or indophenol butanol ethanol (1 : 1) solution (I. B. E.) from the mother liquor mentioned-above, by adding water or 94% ethanol to it, and then they can be easily titrated and discoloured, and moreover, this solution (I. B. W or I. B. E.) is not discoloured by acids.

The indophenol aqueous solution formerly in general use was very unstable, and this was the most serious defect in the indophenol titrimetry, but the indophenol butanol method stated above has at last brought the defect to a close.

The purity of ascorbic acid to be used in the standardization, neither chinoin made ch 929, nor Kahlbaum made of excellent qualities, has ever come up to 100%. Therefore, the result of the standardization must be corrected by determining the real amount of ascorbic acid with iodometry which makes I_2 free from KIO_3 in the presence of KI in sulphuric acid.

Thus determining the real amount of ascorbic acid which decolourizes 1 ml of the above indophenol solution, we calculate the amount of ascorbic acid in sample taking our standard upon the determined value.

We also advocate to use the straight line established between the reciprocal of the titration value taken on the horizontal axis and the concentration of vitamin C taken on the vertical axis as the standard line for determination of vitamin C, with which we can obtain the concentration of vitamin C directly from the titration value.

We believe that this method is convenient for determination of vitamin C, because indophenol-butanol solution is very durable[11].

Numerous studies on the vitamin C in fruits and vegetables have been made, but most of them have been concerned only with the use of these materials as foodstuffs.

A plant produces and consumes vitamins for its own use. Vitamins in plant bodies have physiological significance for life-phenomena of the plant itself.

These significances may be elucidated. With such a purpose we entered on the studies of vitamin C (1936).

Flowers and leaves are those parts of a plant where very important metabolism is operated actively.

Determining vitamin C of flowers and leaves, we noticed that in a flower, petal had more quantity of vitamin C than stamen and pistil or calyx, and that there was interrelation between colours of flowers and quantity of vitamin C[10].

We determined vitamin C of leaves which were not used as foodstuffs, and found the experimental results as in Table 1.

Table 1. Vitamin C Content in Various Flowers and Leaves.

Sample		Vitamin C (mg %)		
		Total	Reduced form	Oxidized form
FLOWERS	Pumpkin	46.16	10.82	35.34
	Lily (white)	44.08	2.44	41.64
	Iris	85.40	68.25	17.15
	Sweet pea (white)	56.00	20.02	35.98
	Pansy (yellow)	133.96	114.82	19.14
LEAVES	Persimon young leaf	846.00	612.40	233.60
	Persimon mature leaf	390.40	271.11	119.29
	Wistaria young leaf	270.03	166.55	103.48
	Wistaria mature leaf	142.33	69.22	73.11
	Gladiolus	310.54	271.11	39.43
	Amaranth	214.93	64.35	150.58
	Rice-plant	107.09	28.23	78.86
	Rape	108.44	32.53	75.91
	Orange juice	41.16	32.00	9.16

Surveying the above results, we generally find more quantity of vitamin C in a leaf than in a flower; a young leaf has much more vitamin C than a mature one. We presume that vitamin C is closely connected with the operation which promotes the growth of plants. We pointed out the interrelation between chlorophyll, plant-catalase and vitamin C.

Then we investigated the relation between sunlight and vitamin C in various fruits and found that the amount of vitamin C in them was nearly in proportion to the quantity of sunlight which shone upon them when they were ripening[12].

Now, we would like to discuss one of the most representative sources of vitamin C in the Orient, the green tea.

Such oranges as lemons and tangerines are generally believed to be abundant in vitamin C, but the amount of vitamin C in the green tea is several times as large as that contained in a lemon or a tangerine.

The green tea is the most important source to supply us Japanese with vitamin C. There are many kinds of the green tea and they vary in vitamin C content. We show the results of our analysis of each kind of the green tea in Table 2.

Table 2. Vitamin C Contents in Green Tea.

Sorts of tea	Vitamin C contents mg. %	Conditions of infusion				Availability of vitamin C (%)	
		Sample g.	Amount of liquid ml.	Temperature of liquid	Time min.	First infusion	Second infusion
Gyokuro	84.4	3	100	60°C	5	49.2	30.6
Matcha	154.2	1	50	(Added hot water and stirred for 1 minute)	—	97.7	—
Sencha	334.4	3	100	100°C	1	53.1	31.1
Kawayanagi*	281.4	3	100	100°C	3	54.1	24.0
Black tea	trace	—	—	—	—	—	—

* Kawayanagi is a green tea of inferior quality.

As you see in Table 2, either Gyokuro (Green tea of the special and superior quality) or Matcha (Powdered green tea of the superior quality) has much less vitamin C content than Sencha (Green tea of the ordinary quality) and Bancha (Green tea of the inferrior quality).

We will explain the reason in the following, and if you take tea in order to supply yourselves with vitamin C regardless of the taste and the flavour, you need not use high-priced kinds.

There is little difference in the manufacturing method between Gyokuro and Sencha, and the disparity in their vitamin C contents is due to the difference in the method of cultivating the leaves of tea as materials.

About twenty days before the harvest time early in May, the plantation of tea is protected from the sun by Sudare (a sunshade made of bamboo) for about ten days, and then, straws are spread over Sudare for several days to shade it from the sun still more completely, and thus, leaves of tea, as the materials of Gyokuro and Matcha which are strikingly green, flavoured and not severely bitter, are picked at the sun-protected plantation.

They call the method "10 days under Sudare and 10 days under straws".

When a plant is protected against the sun while it is cultivated, it tries its best to grow up, by increasing its chlorophyll to use the sunlight to the best advantage and by utilizing fertilizers imbibed through its roots, and accordingly the special flavour and colour of these sorts of tea increase in the leaves.

The amount of vitamin C in a plant is naturally in proportion to the quantity of the sunlight which has shone on the plant, and it was already proved that the amount in the leaves on the tree of tea decreased markedly when they had been wrapped in black paper for only a day to be protected against the sun.

The leaves in the sun-protected plantation contain much less vitamin C than the leaves of Sencha (Green tea of the ordinary quality) cultivated in an open air plantation owing to the quantity of sunlight allowed to shine on them.

As you see in Table 2, black tea has little amount of vitamin C, and this is not due to the method of cultivating the leaves as materials, but it is originated in the manufacturing method.

At the first step of manufacturing the green tea, leaves are steamed for a short time, but as to the black tea, leaves are not steamed at all but they are fermented. This fermentation makes the colour and the flavour of the black tea.

This steaming process aims at killing the so-called oxidase in plant and when it is killed, tea colour and the flavour of the black tea become unchangeable.

Vitamin C in the natural world is easily destroyed by the oxidase, and it is due to the destructive action of the oxidase that leaves and fruits picked off from a plants lose their vitamin C from moment to moment. This is one of the reasons why fresh vegetables and fruits are of great nutritive value.

Raw leaves of the black tea as much vitamin C as the leaves of Sencha, but as they are not steamed, enzyme in them continues to act and makes them the black tea. But, to our regret it is this oxidase in the black tea that destroys vitamin C.

We trust that you have generally understood the reasons of the difference in vitamin C contents between the green tea and the black tea by our explanation stated above.

Formerly we have frequently met with a theory stressed in some books of the nutritional chemistry and of the vitamins that vitamin C is very unstable to heat.

But this theory is quite erroneous. As we have stated above, though vitamin C is very unstable to oxidation, it is by no means unstable to heat,[13)14)] and it stands the heat of steaming process of the manufacturing method of green tea and it is not changed after the leaves of the tea were dried and almost all the amount of vitamin C contained in the raw leaves is kept in the green tea.

We have a column of "percentage of available vitamin C" in Table 2.

We show in this column the percentage of the amount of vitamin C decocted from various sorts of tea.

For example, when we boil 3 grams of Gyokuro in 100 ml of water at 60° C for 5 minutes, we can get a decoction containing 49.2% of the whole vitamin C kept in Gyokuro, i. e. the decoction has nearly one half of the whole amount of vitamin C contained in Gyokuro. Repeating the same means stated-above, we get the second decoction which contains 30.6% of the whole vitamin C kept in Gyokuro.

Thus, we decoct totally about 80 % of vitamin C from Gyokuro by boiling it twice.

Now we see that the first infusion of tea is very high in nutritive value.

Matcha (Powdered tea of the superior quality), like Gyokuro, contains less amount of vitamin C than Sencha as we stated above.

As we do not strain out tea grounds of Sencha but take all of this powdered tea, its availability of vitamin C is much higher than that of other sorts of tea.

Vitamin C in the raw leaves of green tea is scarcely lost throughout the whole manufacturing process, and it is hardly destroyed by boiled water, and serves our nourishment.

Furthermore, green tea contains a considerably large amount of vitamin B_1 and B_2 also, and as a sort of alkaline food, it can prevent acidosis too. So we believe that green tea should not be called a luxurious beverage, but it is an indispensable dried vegetable for the human being.

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