DISTRIBUTION OF ASCORBIC ACID IN RED-SKINNED RADISH AND ITS RELATIONSHIP WITH ANTHOCYANIN PIGMENTS FOR THEIR CO-EXISTENCE

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Introduction

In the exploration of a cheap and rich source of vitamin C in the vegetable kingdom of East Pakistan, which may be used as a supplement to our dietaries, specially to children and pregnant women, it was noted that radish available here is a good source of this vitamin. It is locally called 'Mula' or 'Muli'. There are two varieties of it, one with white skin containing lesser amount of ascorbic acid (24.4 per cent) than the other having pinkish white skin (32.2 per cent), as found on analysis in semi-matured condition.

Both these varieties are eaten in cooked form and as well as uncooked. As the process of cooking destroys to a certain extent the ascorbic acid of both these species, it is preferable to eat it uncooked. It cannot, however, be denied that pink-skinned radish is richer than the white-skinned one, regarding the contents of the above vitamin. From the practical aspect of human nutrition, it will be necessary to study further as to whether the ascorbic acid from this source, even if sufficient in quantity, will be utilised in the human system to the same extent as from citrus fruits like *Citrus decumana*, orange etc. or the crystalline synthetic product as observed by De and Barai[1] and others. However, before taking up this work it was felt desirable to study at what stage of growth of the above plant the yield of ascorbic acid will be maximum and in what way this will be distributed in the pigmented and non-pigmented portions of the root, so that the dietician may properly select the above vegetable at its proper stage of maturity.

The present work therefore aims to determine the distribution of ascorbic acid in pink skined radish in different parts at different stages of maturity. It is also expected to throw light on whether the presence of anthocyanin pigments—pelargonidin and cyanidin[2]—in pink radish has any direct bearing on the high accumulation of ascorbic acid. Although sufficient work has been done to elucidate the relation between ascorbic acid and chlorophyll and the reason for the high accumulation of this vitamin in chlorophyll-containing region of the plants, uptil now our knowledge about the reason for the high concentration of this vitamin in those regions of the plant like skin of the root, flower etc. where pigments of anthocyanin, anthoxanthin and xanthophyll groups are widely distributed in considerable amount, seems to be inadequate.

The independent co-existence of ascorbic acid and chlorophyll was first pointed out by Reid,[3] who had indicated that corolla lacking in chlorophyll, might contain more ascorbic acid than calyx. The observations of De and Baral,[4] Harris and Roy[5] and others that ascorbic acid accumulates in sufficient amount in seedlings germinated in darkness and having thus no chlorophyll, substantiate the above view of independence of vitamin C and chlorophyll, with respect to their association for the performance of some vital enzymatic reactions in the plant kingdom. Giourd[6] on the basis of the findings that fruits, rich in carotene, are also rich in ascorbic acid, attempted to co-relate these two constituents with respect to their co-existence as due to the protective action of carotene against oxidation of ascorbic acid. But the survey of the composition of fruits grown in South America reviewed by Mapson[7] failed to support the above co-relation between carotene and ascorbic acid in metabolic function in the plant kingdom. Thus our knowledge on this entire field is still incomplete and it

will, therefore, be profitable to study the above aspect in greater detail.

The experiments, with pink skinned radish, as detailed below, are expected to throw new light in understanding the relationship between ascorbic acid and the anthocyanin group of pigments for their co-existence in the root of the above plant.

Experimental

The radish used in the present investigation is the commonest one found in East Pakistan and belongs to the species *Raphanus sativus*.[8] In this species the root remains almost colourless at the early stage of growth but gradually the colour of the pigment appears on the upper end adjoining the shoot, spreading downwards with the progress of maturity.

In the present series of work the following batches from three stages of growth of the above species were selected.

(1) *Batch A with average root length of* $2\frac{1}{2}$ *to* 4 *inches.*—In this batch a very faint pink colour developed at the extreme upper end of the root adjoining the shoot.

(2) *Batch B with average length of the root from* 4 *to* 7 *inches.*—In such samples, colour of the pigment occupied almost the entire upper half of the root.

(3) *Batch C with average length of the root from* 7 *inches and above.*—In such samples, the colour of the pigment occupied three-fourth of the entire root surface. The intensity in the pigment colour was, however, deeper at the upper extreme end and faded gradually towards the tip.

From each batch a portion was sliced from the middle of the pigmented and non-pigmented portions, and these were then analysed for ascorbic acid content in triplicate for each sample.

Further, the pigmented skin and white lower fleshy portion beneath the skin from the upper pigmented half of the root of batch B were also analysed for ascorbic acid contents. This batch, because of having both the pigmented and non-pigmented portions in clear distinctive zones, was expected to yield better information about the distribution of ascorbic acid in association with pigment. While going through the literature about the properties of the pelargonidin and other pigments of anthocyanin group, it came to light that this pigment might act as an acid-alkali indicator and may easily be oxidised and reduced.[9,10] Because of its reducing property similar to ascorbic acid, it was necessary to see how far the presence of this pigment in the metarphosphoric acid extract during analysis might interfere with the ultimate titration values of ascorbic acid. In order to make correction due to the presence of the pigment, a new method detailed later was adopted for its estimation.

The green leaves of the shoot with and without stem were also analysed to compare the anthocyanin-associated ascorbic acid content of the skin with the chlorophyll-associated ascorbic acid content of the leaves. Thus, the analysis of the radish as stated gave informations about the distribution of ascorbic acid in various parts of radish of East Pakistan vis-a-vis the values obtained in other countries.

Method of Analysis.—The ascorbic acid contents of the samples were estimated according to the method of Harris and Oliver[11] as well as of Bessey.[12] This was based on the titration of 0.2 c.c of a 0.025% solution of the dye-2:6 dichlorophenol indophenol with the extract of the sample. This extract was prepared by grinding 5 gm. of tissue with a few c.c. of 5% metaphosphoric acid solution having quartz sand; and finally after repeated grinding and centrifugation making up the volume to 25 c.c. The above dye solution was previously standardised against 0.02%

ascorbic acid solution in 5% metaphosphoric acid. Contamination with metals was avoided and all solutions were prepared in water distilled three times.

To determine anthocyanin pigments which also could reduce the dye 2:6 dichlorophenol indophenol like ascorbic acid, a known amount of pigmented skin (2 gm.) was ground with quartz sand in a small quantity of thrice-distilled water, filtered and to the filtrate was added 0.1 N NaOH solution until alkaline. The original pink coloured extract which contained both anthocyanin pigments and ascorbic acid turned blue with the addition of alkali. The whole was then boiled for 10 minutes. By this process the ascorbic acid in the extract was to be completely destroyed. To the extract was then added 5% metaphosphoric acid solution drop by drop until acidic and the pink colour regenerated. The titre value of this extract against the above standard dye solution was then determined according to the procedure described above and from this titre value the reducing property of the pigment towards the dye was evaluated and expressed in terms of ascorbic acid equivalent to mg. per cent of the pigment skin.

Results

The results presented in Table I show that both upper and lower portions of batch A contain moderate amount of ascorbic acid whereas those of batch B contain higher values and those of batch C still higher values. These results apparently indicate that gradual increase in the values in both pigmented and non-pigmented portions may be due to the progress of growth. In order to judge as to whether only the maturity influences the above increase in values with the progress of growth or the appearance of pigment is also partly responsible for this, specially in the case of pigmented upper portion, the data have been analysed in a different way also and presented in Table 2 in which columns (i) and (ii) represented respectively the increase of values of non-pigmented and pigmented portions with the progress of growth; column (iii) indicates the increase of values of pigmented portion over non-pig-

TABLE I.—DISTRIBUTION OF ASCORBIC ACID IN DIFFERENT PARTS OF ROOTS OF PINK SKIN RADISH AT VARIOUS STAGES OF GROWTH.

Sl. Marking	Stage of growth of plant	Length of root (ins.)	Spread of pigment on the root	Portion of root analysed	Ascorbic acid in mg. per cent
A.	Early stage	$2\frac{1}{2}$ to 4	Slight pigment at the upper portion. Rest colourless	Upper pigmented half-A_1	18.7
				Lower non-pigmented half-A_2	15.2
B.	Semi-matured	4 to 7	Upper half pigmented. Lower half colourless	Upper pigmented half-B_1	32.2
				Lower pigmented half—B_2	19.7
C.	Fully matured	7 to 10	Upper $\frac{3}{4}$ portion deeply pigmented	Upper pigmented $\frac{3}{4}$ portion-C_1	43.6
				Lower non-pigmented portion-C_2	21.7

mented portion in each batch, and column (iv) indicates the difference of the increase in values of pigmented over non-pigmented portion due to batch difference.

The values of column (i) thus show that with the progress of maturity there is very little increase of ascorbic acid in the non-pigmented portion even when fully matured (C_2-A_2), whereas the pigmented portion (column ii) shows very high increase with the progress of maturity. From the above values it seems evident that increase in the values of the pigmented portion is not only due to the progress of maturity but may also be due to the appearance of pigment. The influence of maturity, however, is limited only to a small extent and may be best judged from the small increase in values of non-pigmented portion with the progress of maturity. This is further substantiated by the data in column (iii) in which it is noted that whereas the difference in the values between upper and lower portion of batch A, in which the pigment had hardly appeared at that stage of growth, is very small, those in the batches B and C, on the contrary, are very high. Moreover, it is further noted that this influence of the appearance of pigment on the accumulation of ascorbic acid in the pigmented portion maintains almost a constant rate with the progress of maturity as is evident from the values of columns (iii) and (iv) in which it is observed that increase of values of the pigmented upper portions between two subsequent batches i.e. between B_1 and A_1 and between C_1 and B_1 is nearly 11.4 to 13.5 mg. per cent (column ii) and the difference of the increased value of pigmented portion over non-pigmented portion between B and A and between C and B is nearly 9 mg. (column iv) in both cases.

TABLE 2.—EFFECT OF MATURITY AND PIGMENTATION OF SKIN ON THE INCREASE OF VALUES OF ASCORBIC ACID IN RED SKINNED RADISH WITH PROGRESS OF GROWTH.

Increase of ascorbic acid values of non-pigmented portion with progress of growth (*i*)		Increase of ascorbic acid values of pigmented portion with progress of growth (*ii*)		Increase of ascorbic acid values of pigmented portion over non-pigmented portion in individual batch (*iii*)		Difference of increased ascorbic acid values of pigmented portion over those of non-pigmented portion due to different batches (*iv* = *ii*-*i*)	
Difference due to batches and portions	Increased value (in mg. per cent.)	Difference due to batches and portions	Increase of value (in mg. per cent)	Difference due to portions.	Increased of value (in mg. per cent).	Difference due to batches and portions.	Increase of values (in mg. per cent.)
B_2-A_2	4.5	B_1-A_1	13.5	A_1-A_2	3.5	$(B_1$-$B_2)$-$(A_1$-$A_2)$	6.0
C_2-B_2	2.0	C_1-B_1	11.4	B_1-B_2	12.5	$(C_1$-$C_2)$-$(B_1$-$B_2)$	9.4
C_2-A_2	6.5	C_1-A_1	24.9	C_1-C_2	21.9	$(C_1$-$C_2)$-$(A_1$-$A_2)$	18.4

TABLE 3.—DISTRIBUTION OF ASCORBIC ACID IN PIGMENTED SKIN, NON-PIGMENTED FLESH, IN LEAVES WITH AND WITHOUT STEM AND ALSO THE REDUCTION VALUE OF ANTHOCYANIN PIGMENT IN TERMS OF ASCORBIC ACID.

Serial No.	Name of the article	Ascorbic acid values
1	Pigmented outer skin of Batch B ..	41.6 mg. per cent of skin.
2	Non-Pigmented flesh beneath the skin of Batch B	28.5 mg. per cent of flesh.
3	Leaves with stem	80 mg. per cent of the whole stalk with leaves.
4	Leaves without stem	50.0 mg. per cent. of the leaves.
5	Pigment extract of the skin	Equivalent to 1.5 mg. per cent of the skin.

The above results thus clearly indicate that ascorbic acid is concentrated in greater amount in the pigmented upper portion. The pigmented skin removed carefully by glass edge from the surface of the upper pigmented portion of sample B showed higher ascorbic acid content than the white portion just beneath the skin (Table 3) and these results further indicate that in the upper pigmented portion the ascorbic acid is concentrated in association with anthocyanin pigment. The possibility that the above high ascorbic values of pigmented portion may also be due to reduction of 2:6-dichlorophenol indophenol dye by the pigment similar to ascorbic acid has been corrected by separate estimation of the pigmented extract after destruction of ascorbic acid and it has been observed that the reduction value of the above dye by the pigment has an ascorbic acid equivalent of 1.5 mg. per cent of the skin (Table 3).

Kihara and Kimura[13] studied the ascorbic acid content in different parts of the round edible root of radish and found the values of 19·6, 17·5, 16·1, 18·5 and 18·5 mg. per cent for the upper, middle and lower parts and also for outer and inner parts respectively. In contrast to the findings of the present investigation, the above values of Kihara and Kimura do not show any significant difference regarding their distribution in different parts of the root and this may presumably be due to the equidistribution of the pigment all over the round root. Also it is noted that the maximum value of any part of their species almost approaches the minimum value recorded in the present studies. The range of values of the roots in this investigation conforms to those submitted by the U.S. Department of Agriculture[14] (15-40 mg.) and by F.A.O.[15] (13 to 30 mg.).

Analysis of the green leaves with and without stem showed the ascorbic acid values of 80 and 50 mg. per cent respectively (Table 3). Similar results were also submitted by Japanese and other workers.[13,16]

Discussion

While discussing the above results of high concentration of ascorbic acid in association with anthocyanin in the pink radish root, one needs review the function of ascorbic acid in the plant kingdom. In every organism, ascorbic acid by virtue of its possession of oxida-

tion-reduction potential plays an important role in the respiratory mechanism, as a terminal step for direct oxidation of metabolite by oxygen in association with a large number of enzymes and carriers.[7,17,18] In the plant kingdom this property of ascorbic acid is not only limited to the above process but also extends to photosynthetic mechanism in the process of hydrogen transfer. The reports by different workers[19-26] that most of the enzymes and carriers of terminal oxidation system of respiratory mechanism are present in chloroplast in association with chlorophyll and ascorbic acid, combined with the observations that chlorophyll[27-28] can be reversibly and photochemically reduced by ascorbic acid and further that ascorbic acid participating in Mehler Reaction helps in the consumption of oxygen[29] have opened new chapters in understanding the cause of association of ascorbic acid with chlorophyll for photosynthetic reaction. But on careful survey of the data presented here, it becomes clearly evident that the amount of ascorbic acid concentrated in association with the anthocyanin pigment on the skin (41·6 mg. per cent) does not seem to be very low, as compared to that in the leaves associated with chlorophyll (50 mg. per cent). It is thus reasonable to assume that anthocyanin complemented with ascorbic acid perhaps performs similar function as that by chlorophyll in association with the above vitamin in the leaves. The above phenomena of co-existence of anthocyanins and ascorbic acid in the root may be explained on the basis of the property of these pigments in being easily oxidised and reduced for which they may act as hydrogen acceptors and oxygen carriers as viewed by Frear.[10]

(1) Metabolite + (DPN or TPN) —*Dehydrogenase*→ Oxidised Metabolite + (DPNH or TPNH)

(2) (DPNH or TPNH) + Anthocyanin (AN) —Anthocyanin Reductase ?→ (DPH or TPN) + ANH

(3) (ANH + DAA) —Dehydroascorbic acid Reductase→ N A + AA

(4) AA + ½ O_2 —Ascorbic acid oxidase→ DAA + H_2O

With this background of informations in our hand it may be hypothesised that the above two constituents of the root, *viz.*, anthocyanin pigments and ascorbic acid, along with some enzymes and carriers may form a chain of respiratory system for terminal oxidation. One such scheme of reaction mechanism based on the transfer of hydrogen from the metabolite by dehydrogenase through DPN and TPN may be postulated as below:—

According to this scheme hydrogen will be transferred to anthocyanin (AN) from DPNH or TPNH (React. 2) and this perhaps occurs by the participation of a hitherto unkown enzyme (may be termed as Anthocyanin reductase?). In the next reaction reduced anthocyanin (ANH) will transfer the hydrogen to dehydroascorbic acid by the help of the enzyme dehydroascorbic acid reductase. Thus AN will be regenerated in the above way. In the next step hydrogen will be transferred to oxygen from ascorbic acid (AA) either by the participation of ascorbic acid oxidase or other enzymes and carriers. The possibility of the participation of anthocyanin in the above scheme of terminal oxidation gets support from the observation of Thomas[30] that oxygen is always absorbed when anthocyanins are formed in healthy plants. Reviewing the above scheme it is not unusual to assume at this stage that AN will thus protect AA from oxidation according to the reversible process of Reaction[3] in the same manner as glutathione (GSH) does.[31,32] This offers an explanation as to the high content of ascorbic acid in the species of radish having pink root.

Further work is now in progress to get some information as to whether the biosynthesis of ascorbic acid in the roots depends on anthocyanin groups of pigments independent of chlorophyll and vice versa and this pigment in association with ascorbic acid may independently perform photosynthetic mechanism like that by chlorophyll in leaves.

From what has been discussed in this article we are led to the conclusion that it is quite safe to recommend to the consumers of radish in this country that it will be more useful if they make it a habit to eat the outer skin of the radish uncooked as a sort of salad together with the young leaves. This will help supply them with a fair quantity of vitamin C, which they need in their daily diet. The present observations should give a warning, to those who are in the habit of throwing the skin of radish, that they are doing a great wastage of ascorbic acid from the vitamin resources of their dietaries.

Summary.

Analysis of the Red-skinned rat-tail radish grown in this region showed that the ascorbic acid contents of the root in pigmented and non-pigmented portions increase with growth. At all stages of growth the ascorbic acid content was found to be higher in pigmented portion. Separate estimation of the pigmented skin and the white flesh beneath the skin showed that this acid was concentrated more in association with the anthocyanin-group of pigments in the skin. The significance of these results with respect to close association of ascorbic acid with anthocyanin in the skin for the participation in some terminal oxidation process in respiratory mechanism, has been discussed in this paper.

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