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Oxidizing Enzymes and Vitamin C in Tomatoes

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Sixteen years ago Willimott & Wokes [1926 *a, b, c*] studied the occurrence of oxidizing enzymes and vitamin C in the peel and juice of citrus fruits. The enzymes were found to be present in highest concentration in the rind. When lemon rind was being assayed for vitamin C, in order to avoid destruction of the vitamin by these enzymes, the rind was cut into small pieces after removal, weighed, and mixed with 90% ethanol to prepare a tincture for administration to guinea-pigs. This tincture failed to prevent scurvy when administered as the sole source of vitamin C in daily doses equivalent to 0.5–1 g. of the rind, although the onset of symptoms was slower than in negative controls, so lemon rind was assumed to contain only slight amounts of the vitamin as compared with the juice, which has a considerable amount.

Later, when a satisfactory chemical test for vitamin C had become available, this was applied by Bacharach, Cook & Smith [1934] to orange and lemon rinds. Orange rind, which Willimott & Wokes [1926*c*] had reported to contain only moderate amounts of the vitamin, was found to contain a concentration of vitamin C several times that of the juice, whilst lemon rind also contained more than lemon juice. Bacharach *et al.* [1934] suggested that the discrepancy between their results and those of Willimott & Wokes might be due to loss of vitamin during preparation of the tincture, but made no reference to the oxidizing enzymes. Willimott & Wokes had drawn attention to these enzymes, but assumed that the use of 90% ethanol, in which the enzymes would not be soluble, would prevent their destroying the vitamin. Only within the last few months has it been realized that when these enzymes are set free by mincing the tissues a considerable amount of the vitamin may be destroyed in a few minutes.

In the interval between the appearance of the papers of Willimott & Wokes [1926 *a, b, c*] and that of Bacharach *et al.* [1934] Szent-Gyorgyi [1931] showed that his 'hexuronic acid' was rapidly destroyed by an oxidizing enzyme when the inner leaves of cabbage were minced. Qualitative tests

indicated that most of the 'hexuronic' acid had disappeared within 5 min. of completion of mincing. The enzyme was named hexoxidase because it attacked hexuronic acid, but since the latter is now known as ascorbic acid the enzyme is now known as ascorbic acid oxidase.

Kohman, Eddy & Gurin [1931] reported that minced carrots lost much of their antiscorbutic activity on standing in air for 1 hr., but biological assays did not prove suitable for investigating this problem. Zilva [1934], using both biological and chemical methods, showed that apple juice contains an enzyme which destroys the vitamin C in lemon juice, approximately 84% being lost at pH 4.4 and 57% at pH 3.0, both in 6 hr. at room temperature, and that, as might have been deduced from the findings of Willimott & Wokes, the juice is a less potent source of the enzyme than the peel. However, during the following 8 years most workers on this enzyme neglected fruit and concentrated on vegetables. Thus Ahmad [1935] showed by chemical assays that 11% of the vitamin C in karela was lost in 10 min. and 17% in 20 min. when this common Indian vegetable was shredded. McHenry & Graham [1935] obtained similar results with shredded turnips, parsnips and cauliflower. Tauber, Kleiner & Mishkind [1935] found the enzyme in *Cucurbita maxima* and named it ascorbic acid oxidase. Hopkins & Morgan [1936] extracted the enzyme from cauliflower florets and showed that the enzyme extract would destroy about 70% of added ascorbic acid in 5 min. at pH 6 and 18°. It was apparent from their results that if the ratio of enzyme to ascorbic acid had been similar to that which occurs naturally even more rapid destruction of the vitamin would have occurred. Kertesz, Dearborn & Mack [1936] showed that when cabbage was ground with an equal weight of water it lost about 97% of its ascorbic acid content in 1 hr. and reported similar results with other vegetables. Johnson & Zilva [1937] expressed the juice from frozen cabbage, cauliflower, cucumber and marrow but found that the destruction in 1 hr. of ascorbic acid added to cauliflower juice was only 33% at pH 5 and 35% at pH 7, much slower than the rate found by Hopkins & Morgan [1936]. They confirmed Hopkins & Morgan's findings, however, that the optimum pH for the enzyme is about 6, and showed that at pH 3.0 the activity was very slight, but at pH 9.0 still fairly pronounced. Stone [1937] minced vegetables containing vitamin C, and showed that in cabbage juice a loss of 98% of free ascorbic acid and about 63% of total ascorbic acid occurred in 5 min. Like Kertesz *et al.* [1936] he found that the rate of destruction in spinach was much slower. Lampitt & Baker [1942] observed that when orange

peel was minced and allowed to stand, 17% of the ascorbic acid was destroyed in 3 hr. and over 90% in 22 hr. Similar results were obtained with cabbage. The destruction of the vitamin was attributed to an oxidase. Pyke [1942] showed that the rate of destruction was increased by dividing the tissue more finely and thus liberating the enzyme sooner. In finely shredded cabbage as much as 32% of the vitamin was destroyed in 5 min., but in swedes the rate of destruction was lower. Lampitt, Baker & Parkinson [1942] confirmed and extended Pyke's results, finding a 40% loss in 1 min. and a 60% loss in 7 min. Wokes & Organ [1942] found similar losses in minced green tomatoes.

EXPERIMENTAL

Ascorbic acid oxidase appears to be present in highest concentration in green vegetables and in the skin of fruits. Ebihara [1938] reached the same conclusion. We selected tomatoes for our experiments, obtaining them from three sources: King's Langley (outdoor), Elstree (greenhouse), Wilmslow, Cheshire (outdoor and greenhouse). Vitamin C was estimated by the dye micro-titration method as developed by Harris & Olliver [1942], using precautions described below. Some results on coloured solutions were checked by the potentiometric method of Harris, Mapson & Wang [1942]. Tests for oxidizing enzymes were applied as described by Willmott & Wokes [1926b] both to the tissues themselves, and to aqueous extracts prepared by grinding the tissues in a glass mortar with a special fine grade of quartz sand made by Mr A. L. Curtis of Chatteris. pH determinations were made with the glass electrode.

Oxidizing enzymes and pH

In unripe tomatoes oxidizing enzymes occur in the highest concentration in the skin; the flesh and seeds contain less, and the juice very little. In ripe tomatoes the concentrations appear to be similar to those in unripe tomatoes, but slightly lower.

The pH of the skin of unripe tomatoes was found to be high, ranging from 8.5 to 9.6 in samples examined within a few days of picking. When green tomatoes are kept for several weeks the pH of the skin may, under certain conditions, fall slightly, but if ripening proceeds normally the skin seems always to remain alkaline. In making pH determinations on the skin of ripe tomatoes it is important to remove all the flesh, which is acid.

The juice of tomatoes appears always to be acid. That of unripe tomatoes ranged from pH 3.9 to 5.0 (average 4.4), and of ripe tomatoes from pH 4.2 to 4.5. In the flesh the reaction changes from alkaline to acid during ripening. In firm green unripe tomatoes the pH of the flesh ranges from 8.4 to 9.0. When such tomatoes are ripened after picking either in the dark or in the light the pH gradually falls to between 4.6 and 5.5, a range similar to that of the flesh of tomatoes ripened on the plant.

These findings suggested that the rate of destruction of vitamin C by ascorbic acid oxidase would be most rapid in the skin, where the concentration of oxidase is highest and the pH most favourable, and slowest in the juice, where the reverse conditions prevail. When whole tomatoes are minced the pH of the mince depends largely on that of the flesh. Since the flesh is alkaline in unripe, and acid in ripe tomatoes, the rate of destruction of the vitamin in minced whole tomatoes should be more rapid in the unripe. These suppositions have been verified.

Rate of destruction of ascorbic acid in different parts of the tomato

The precautions adopted are as follows: the skin was removed in small pieces with a stainless steel knife from four green tomatoes of similar weight and from the same source. Each piece was immediately either immersed in a freshly prepared solution of HPO_3 containing 25 g./100 ml. in a tared weighing bottle or alternately in one of two empty weighing bottles labelled respectively 'control' and 'experimental'. The three bottles were weighed to determine the amount of skin in each; the skin from one of the empty weighing bottles was transferred quantitatively to a glass mortar with quartz sand and distilled water. At a given time grinding was begun, and it was continued for the required time, say 5 min., further quartz and water being added as necessary. Exactly 5 min. after grinding commenced HPO_3 solution was added to stop the action of the oxidase; the contents of the mortar were pressed through muslin; the mass was returned to the mortar and extracted twice more with acid and water, and the combined extracts and washings made up to 100 ml. At the start of the grinding HPO_3 solution had been added to the control. The contents of the control bottle and of the bottle which initially contained acid were extracted in the same way, each being made up to 50 ml. and the ascorbic acid content determined. The concentration of ascorbic acid in the skin of the control was assumed to be the same as that in the experimental skin when grinding commenced. This procedure is necessary because with skin an appreciable amount of ascorbic acid may be lost merely on standing, through the action of oxidase liberated when the skin is removed. In the above experiment 19% of the initial ascorbic acid was lost in the skin whilst standing for 30 min. Of the remainder, 98% of the free ascorbic acid and 95% of the total ascorbic acid were destroyed during the 5 min. grinding.

There are several possible sources of error in this technique, but consistent results were obtained in a series of 26 experiments, summarized in Table 1. The rate of de-

Table 1. *Rate of destruction of vitamin C by oxidase liberated on grinding different parts of tomatoes with quartz sand*

Period of grinding min.	Skin		Flesh		Whole	
	Unripe %	Ripe %	Unripe %	Ripe %	Unripe %	Ripe %
1	67	47, 50	—	—	—	—
2	69	76	38	26	26, 28	—
3	84	—	—	—	—	6
5	98	99	—	—	—	27
10	—	—	64	48	—	—
12	—	—	Juice		—	29
15	—	—	64	—	95	33
20	—	—	—	19	—	—
30	—	—	—	—	—	37
60	—	—	—	—	—	45
120	—	—	—	14	—	71
20 hr.	—	—	—	—	—	95

The results represent percentage of vitamin C destroyed by grinding for the given time.

All results obtained on the juice and whole tomato included seeds except the 120 min. sample of ripe juice.

struction was most rapid in the skin and slowest in the juice. When the seeds were removed from the juice before grinding the rate of destruction of the vitamin was still further reduced because of the removal of the seed oxidase. When whole tomatoes were ground with quartz sand or minced, the rate of destruction was more rapid in unripe tomatoes, and intermediate between that for the skin and that for the juice. Moreover, when green tomatoes were sliced the rate of destruction of the vitamin decreased as the slices were cut coarser [Wokes & Organ, 1942]. All these findings suggest that the rate of destruction of the vitamin depends on the concentration of oxidase in the tissues and the rate at which this is liberated by mechanical disruption of the cells.

When weighed amounts of skin were ground with quartz and a known amount of ascorbic acid in phthalate buffer at pH 4 the rate of destruction of the ascorbic acid became slower as the ratio of oxidase to ascorbic acid was decreased. When the relation between the oxidase and the ascorbic acid was such as would occur when the skin of a tomato was ground with the whole of the flesh and juice, the rate of destruction was similar to that found when grinding whole ripe tomatoes (e.g. 20% destroyed in 5 min., 45% in 30 min.). This was further confirmation of the validity of the above theory.

Sampling of tomatoes

If the pieces of tomato are too large and skin is present, there may be destruction of the vitamin inside the tissue before the acid can reach and inactivate the enzyme. We used samples of 3–10 g. for whole tomatoes or their flesh, and 0.5–3 g. for the skin. These samples were cut up into small pieces under HPO_3 solution, using a stainless steel knife, and then ground with the quartz sand. Since only a portion of the tomato is used for each assay this raises the question of sampling. As will be seen later, the concentration of vitamin C may vary by several hundred % in different parts of the same tomato. The accuracy of our sampling procedure was tested by analysis of replicate samples, and determining the percentage standard deviation or coefficient of variation of a single assay result. The average results thus obtained on over a hundred samples are summarized in Table 2. Apart from the incubated

Table 2. *Sampling errors in vitamin C estimations on tomatoes*

Part of fruit assayed	Coefficient of variation for the estimation of vitamin C in a single sample		
	Unripe	Ripe	Incubated
Whole	5.8	5.1	8.8
Skin	3.1	3.6	7.2
Flesh and juice	8.2	7.8	—

tomatoes, the results from which are subject to special errors to be considered later, these results indicate that in sampling whole tomatoes our average coefficient of variation for the mean of two assays was about 4. When comparing results on the same tomato we therefore considered a difference of 8% significant ($p=0.95$). Very little information is available regarding the sampling error when estimating vitamin C. Olliver [1938] quotes results on duplicate samples of strawberries giving coefficients of variation of 6.1 for unripe and 2.9 for ripe red fruit, which

are of the same order as our own sampling errors for tomatoes.

We also considered how much of the so-called sampling errors might be due to titration errors. At least four titrations were done on each sample, the end-point usually being checked by two observers. From the results of many hundreds of titrations we concluded that differences between two assay results due to titration errors would be not more than 2%. When titrating extracts of ripe tomato skin we removed the pink colour by centrifuging. The visual results were also checked by parallel determinations on different samples by the potentiometric method of Harris *et al.* [1942], which gave results agreeing closely with visual results, the average coefficient of variation between the two methods being 1.7 in assays on 12 samples.

RESULTS

Concentration of vitamin C in different parts of the tomato

Our results (Tables 3 and 4) for the estimation of ascorbic acid in the different parts of the flesh, and

Table 3. *Vitamin C concentration in different parts of ripe tomatoes*

Wt. g.	Ascorbic acid (mg./100 g.)				Distribution ratio (skin/whole)
	Whole	Skin	Flesh	Juice	
35.4	13.7	37.9	—	—	2.76
36.2	15.2	30.0	—	—	1.97
40.6	17.6	30.1	14.9	10.7	1.70
44.8	17.9	62.4*	—	—	—
55.9	17.3	25.6	—	—	1.48
56.9	16.0	36.2	11.0	16.9	2.26
61.5	19.2	42.6	—	—	2.22
63.0	19.5	42.2	—	—	2.16
76.6	18.7	42.7	14.1	12.6	2.28
83.4	—	29.4	—	18.2	—
Mean	16.9	35.2	13.3	14.6	2.10
S.D. of mean	1.9	6.6	—	—	0.39
Coefficient of variation	11.5	18.7	—	—	18.6

* This skin was cut off more finely than usual, and the result is not included in the mean.

Table 4. *Vitamin C concentration in different parts of unripe tomatoes*

Wt. g.	Ascorbic acid (mg./100 g.)				Distribution ratio (skin/whole)
	Whole	Skin	Flesh	Juice	
6.9	12.0	23.9	—	—	1.99
7.8	12.9	20.2	—	—	1.58
30.5	10.0	18.3	11.9	8.5	1.83
52.2	23.4	50.0	—	—	2.14
53.3	13.4	23.1	—	—	1.72
58.2	14.9	23.0	—	—	1.54
58.6	15.5	33.9	8.3	17.7	2.16
59.0	18.1	34.0	—	—	1.88
Mean	15.0	28.3	10.1	13.1	1.86
S.D. of mean	4.1	10.5	—	—	0.23
Coefficient of variation	27.3	37.1	—	—	12.4

in the juice, of ripe and of unripe tomatoes, are, we believe, on the basis of our experiments described above, free from errors resulting from oxidase destruction of the vitamins during the separation of the different tissues.

There were wide variations from one tomato to another and the coefficients of variation were much greater than those given in Table 2, showing that the variation in vitamin C content of different tomatoes was not due to sampling errors. Nevertheless, in any given tomato the concentration of vitamin C in the skin was always definitely greater than that in the whole fruit. The distribution ratio (ratio of concentration in skin to that in the whole fruit) ranged from 1.48 to 2.76 (mean 2.10) in ripe tomatoes and from 1.54 to 2.16 (mean 1.86) in unripe tomatoes. The coefficients of variation showed that in unripe tomatoes there was much less variation in this distribution ratio for the skin than in the individual figures for skin and for whole fruit, showing that the factors affecting the vitamin C content of the skin also affected that of the whole fruit. Our data (Table 3) on ripe tomatoes did not show this so clearly, possibly because of variations in the conditions of ripening, of which more will be said later.

Only a few samples of flesh and juice were examined. The vitamin C concentration in these is always much less than in the skin. The mean distribution ratios did not show any significant difference in vitamin C concentration between the flesh, juice or whole fruit. There was also no significant difference between the vitamin C concentrations in different parts of the flesh. On the other hand, the seeds both of ripe and unripe tomatoes contain a much lower concentration of the vitamin than the whole fruit. We were not able to estimate the vitamin C accurately in the seeds of a single tomato, the estimation of which is required in order to calculate individual distribution ratios, but data obtained on pooled samples from a number of tomatoes indicated that the mean distribution ratio for seeds is about 0.2. As the seeds form only about 1% of the total weight of the fruit their contribution to its vitamin C value is insignificant.

Table 5. *Mean distribution ratios for vitamin C concentrations in different parts of tomatoes*

	Wt. of part % of whole	Distribution ratios	
		Unripe	Ripe
Skin	3-4	1.86	2.10
Flesh	} 95-96 {	0.87	0.76
Juice		1.00	0.78
Seeds	1	0.2	0.2

Distribution ratio for any given part represents concentration of vitamin C in that part divided by the concentration in the whole tomato from which the part was taken.

In Table 5 are given the mean distribution ratios for vitamin C in different parts of the tomato. These show a steady drop in the concentration of the vitamin as one proceeds from the skin inwards. The concentration in the skin is 2 or 3 times that in the flesh and juice, and about 10 times that in the seeds. This applies equally to unripe and ripe fruit.

Factors affecting vitamin C content of tomatoes

(a) *Weight and polyploidy.* Since the concentration of vitamin C in the skin is 2 or 3 times that in the flesh and juice, and since the proportion of skin to total weight of fruit decreases as tomatoes become larger, the concentration in the whole fruit should diminish as tomatoes became larger. This was predicted by McHenry & Graham [1935] who suggested that it would explain why Sansome & Zilva [1933] had found a higher concentration of vitamin C in tetraploids than in diploids, the former being on the average smaller than the latter. Sansome & Zilva [1936] gave further data supporting their previous findings in regard to polyploidy and its effect on the vitamin C content, and did not accept the suggestion of McHenry & Graham that the weight of the tomatoes might be a deciding factor. None of these workers, however, gave the data for the relative concentrations of the vitamin in the skin and other parts of the tomato which were essential to test the validity of the argument. Rudra [1936] found 57 mg./100 g. in the skin of Indian tomatoes, but as the flesh of these tomatoes contained 39 mg./100 g., much more than was found in British tomatoes either by Sansome & Zilva or by ourselves, Rudra's results are not applicable to the problem of polyploidy.

We found that in tomatoes weighing not less than 20 g. the skin consisted of less than 4, and usually less than 3%, of the total weight. If the concentration of vitamin C in the skin is 3 times that in the flesh or juice, an increase from 3 to 6% in the proportion of skin would cause an increase of only 6% in the concentration of the vitamin in the whole fruit (i.e. the concentration in the latter case would be 106% of that for the former).

The difference in vitamin concentration between diploids and tetraploids observed by Sansome & Zilva [1933] was about 68%, i.e. average content for diploids 22 mg./100 g. and for tetraploids 37 mg./100 g. This was a much greater value than the 6% which we calculate would be produced by doubling the proportion of skin. Actually the proportion of skin in Sansome & Zilva's tetraploids (mean wt. 13 g.) would be less than twice that in their diploids (mean wt. 42 g.). We therefore consider that the variation in weight in Sansome & Zilva's tomatoes made no significant difference to their conclusions regarding polyploidy.

The results of estimations on 29 unripe and 20 ripe diploid tomatoes all grown under the same conditions are summarized in Table 6, and show that in unripe tomatoes

Table 6. *Vitamin C concentration in tomatoes of different weights*

No. of samples	Range in wt. g.	Mean wt. g.	Vitamin C (mg./100 g.)		
			Range	Mean	S.D. of mean
Unripe					
5	0.3-11	6.6	10.6-33.3	25.0	4.0
5	18-26	22	12.6-28.8	21.2	2.8
9	30-47	39	10.5-20.7	15.1	1.5
10	50-87	62	13.1-27.3	17.7	
Ripe					
20	36-83	52	11.2-21.4	16.7	0.9

weighing 0.3-11 g. the average concentration of vitamin C was about 34% higher than in unripe tomatoes weighing 30-87 g. However, the difference was scarcely significant because of the wide variation in vitamin C content between tomatoes of similar weights. Hence, in order to obtain a conclusive answer regarding the effect of polyploidy on vitamin C concentration it will be necessary to control other factors affecting the vitamin content in order to reduce the variations we have disclosed. The importance of these factors was recognized by Sansome & Zilva [1936] in a further examination of the problem. We might add that when examining another series of diploid tomatoes grown in a greenhouse at Bushey we found no significant difference in vitamin C concentration for a weight range of 7-59 g., and we are inclined to think that the difference of 34% observed in our King's Langley tomatoes was rather above the average.

(b) *Conditions of ripening.* We were not able to find a significant difference in mean vitamin C concentration between unripe and ripe tomatoes. Thus 21 unripe tomatoes weighing 18-87 g. (average 41.2 g.) had an average content of 17.1 mg./100 g. as compared with an average of 17.5 in 15 ripe tomatoes weighing 41-77 g. and averaging 51.7 g. These tomatoes were all grown under the same conditions. Green tomatoes collected from the same plant growing out of doors at King's Langley were divided into three balanced sets of ten. One set was ripened in sunlight, the second in the dark at room temperature, and the third in an incubator at 37°. As the tomatoes became ripe they were assayed for their vitamin C content with the precautions suggested above. Table 7 shows that the vitamin C content of those ripened in sunshine averaged 17.7 mg./100 g. (similar to tomatoes ripened on the plant); that those ripened in the dark averaged only 12.9, and that those ripened in the incubator averaged only 8.5. Also the tomatoes ripened in sunshine tended to increase in vitamin content as ripening was prolonged, while those in the dark showed a gradual loss of vitamin after the first few weeks, especially at 37°.

Table 7. *Effect of sunlight and heat on vitamin C content of ripening tomatoes*

Period of ripening days	mg. ascorbic acid/100 g. tomato Conditions of ripening		
	In 'sunlight' at room temp.	In dark at room temp.	In dark at 37°
2	—	—	11.1
6	—	—	12.7
8	11.4	—	10.0
11	—	12.7	—
14	16.0	17.6	13.4
20	18.7	14.6	8.4
20	—	17.3	—
22	—	15.4	—
22	17.6	11.2	—
27	17.9	8.8	6.2
28	19.2	12.1	—
31	14.9	—	6.5
32	13.4	—	6.4
35	19.5	10.3	—
37	—	9.4	—
42	28.5	—	2.3
Mean	17.7	12.9	8.5

Results are all calculated on initial weights of tomatoes (i.e. at beginning of ripening period). During this period there were gradual losses in weight, especially in the tomatoes ripened at 37°, in which the loss reached 30% after 4 weeks.

Since these results indicated that exposure to sunlight affects the vitamin C content we tried the effect of exposing tomatoes to ultra-violet light during ripening. Green tomatoes from King's Langley were irradiated daily by exposure to a mercury vapour lamp at a distance of 1 ft. This provided no appreciable amount of radiation below 250mμ and we do not think it was destroying any of the vitamin. In order to diminish the heating effect, the lamp was switched off for a few minutes every 10 or 15 min. The total amount of irradiation was 1 hr. on the first day, gradually increasing to 2 hr. on subsequent days. Unfortunately the irradiation did not produce normal ripening, the skins becoming deeply wrinkled and the flesh being softer than usual. The mean vitamin C content in the tomatoes after 4-7 days' irradiation was 16.9 mg./100 g., no evidence being obtained of an increase in vitamin content. Brown & Moser [1941] found the concentration of vitamin C in tomatoes grown in greenhouses to be only half that of tomatoes grown out of doors. Our data support these findings: five grown in the open had an average content of 15.2 mg./100 g. as compared with 12.6 for five grown in a greenhouse. The number of samples examined was, however, not large enough to make this difference significant.

While exposure to sunlight may increase the vitamin C content of tomatoes, we attribute some at least of the low results we obtained on tomatoes kept in the dark for 5-7 weeks to enzyme action. These tomatoes showed a softening of the flesh immediately beneath the skin which probably allowed the ascorbic acid oxidase to gain access to the juice. Other workers have reported increases in vitamin C content when tomatoes were ripened to a soft condition in sunlight, but our degree of softening was probably greater. A tomato which had been ripening in the sunlight for 7 weeks had a slight bruise extending from just beneath the skin towards the centre. When taking

samples we avoided the bruised part, and our two samples gave results of 27.4 and 29.5 mg./100 g. Two further samples taken from parts in contact with the bruise, but not including it, gave results of 11.3 and 14.5. In our opinion these results indicate the possibility of a rapid loss of vitamin C in bruised tomatoes by the penetration of the oxidase into the juice. The tomato appeared perfectly normal apart from the bruise.

DISCUSSION

Although tomatoes are not the richest natural source of vitamin C they possess advantages over the green leafy vegetables and small fruits such as blackcurrants and rose hips in studies relating to this vitamin. One of the most pressing problems, as pointed out by Bacharach *et al.* [1934], is to explain the wide variations in vitamin C content found in different samples of a given fruit or vegetable. Bacharach *et al.* [1934] worked with citrus fruits, big enough to permit the different tissues to be examined separately, so that the distribution of the vitamin could be studied. If Bacharach *et al.* [1934] had correlated their results with the previous findings of Willmott & Wokes on pH and oxidases in citrus fruits, they would not only have explained the low results obtained by Willmott & Wokes for vitamin C in citrus peels, but also have provided information of general interest on vitamin C in relation to ascorbic acid oxidase. During the present shortage of citrus fruits in this country tomatoes form an excellent alternative for investigation. We were able to study the occurrence of vitamin C and ascorbic acid oxidase in different tissues, and to evaluate errors due to sampling and other causes. Lack of such knowledge must affect the validity of conclusions drawn by previous workers.

In the short season during which home-grown tomatoes are available conclusive evidence could not be obtained on all the points we investigated. The effect of weight and polyploidy on vitamin C concentration was left uncertain. Our failure to confirm the findings of Brown & Moser [1941] that tomatoes ripened out of doors contain twice as much vitamin C as those ripened in a greenhouse was perhaps due to the vagaries of the British climate, for we have little doubt that our out-of-door ripened tomatoes received much less sunshine than those of Brown & Moser. This lack of sunshine may explain why our tomatoes contained less vitamin C than other workers have found in tomatoes grown in warmer climates. The only other results we could find for British grown tomatoes were those of Sansome & Zilva [1933], whose average figure of 22 mg./100 g. for diploids was higher than our figures of 15–18, but lower than McHenry's [1935] figures of 18–36 for Canadian tomatoes, and considerably lower than the figure of 39 obtained by Rudra [1936] for Indian tomatoes.

In rose hips Melville & Pyke [1942] found very much higher concentrations of vitamin C in species native to the north of England and Scotland than in those of the south of England. Their findings, which were obtained on a large number of small samples, have been confirmed on large-scale samples comprising several tons of hips by Wokes, Johnson, Duncan, Organ & Jacoby [1942], and in other unpublished results. Sabalitschka & Priem [1941] reported that hips growing on the north side of a bush contained more vitamin C than those growing on the south side of the same bush. All these findings suggest that the effect of sunshine on vitamin C formation in plants needs to be considered.

The effect of oxidizing enzymes on vitamin C also merits further investigation. Our results show that if this is not allowed for there may be serious losses of the vitamin. We have provided some evidence that when tomatoes are ripened and stored under conditions favouring enzyme action the oxidase may destroy much of the vitamin. On the other hand, the highest concentrations of vitamin C are usually found in tissues containing the largest amounts of oxidase, suggesting that under certain conditions the action of the oxidase may be reversed, and synthesis of vitamin C take place. In this connexion our findings on the occurrence of oxidase in tomato seeds are of interest, since the seeds when examined contained no significant amount of vitamin C, but the latter could be formed by germinating the seeds in the dark.

In our opinion exposure to sunshine and contact with ascorbic acid oxidase will prove to be of much greater importance than factors such as polyploidy in determining the vitamin C value of foods.

SUMMARY

1. Oxidizing enzymes occur in highest concentration in the skin of both ripe and unripe tomatoes. The flesh and seeds contain less enzyme and the juice practically none.

2. The skin is markedly alkaline (pH 8.0–9.6) in both ripe and unripe tomatoes. The reaction of the flesh changes from the alkaline (pH 8.4–9.0) to the acid (pH 4.6–5.5) side during ripening. The juice appears always to be acid (pH 3.9–5.0).

3. When oxidase is set free by mechanical disruption of the tissues it destroys vitamin C. The rate of destruction is most rapid in the unripe skin (e.g. 67% destroyed in 1 min.), less rapid in the flesh and slowest in the juice, paralleling the oxidase concentration and pH in these tissues.

4. The distribution of vitamin C usually runs parallel with the oxidase concentration, being 2 or 3 times higher in the skin than in the juice or flesh. The higher concentration of oxidase but lower concentration of vitamin C in the seeds may be related

to the fact that vitamin C can be produced in the seeds by germination.

5. Because of the variation in vitamin C concentration in different tissues and the action of oxidase during dissection, the sampling error is considerably higher than the normal titration error, and if this is not taken into account it may seriously affect the results.

6. Very small tomatoes may, because of the higher proportion of skin, contain higher concentrations of vitamin C, but the differences found in tomatoes grown under the same conditions were not large enough to discredit the findings of Sansome &

Zilva on polyploidy, as McHenry & Graham had suggested.

7. Exposure to sunshine during ripening may affect the vitamin C content of tomatoes, but the latter is also affected by the oxidase, which may destroy a considerable amount of the vitamin if ripening takes place under conditions favouring oxidase action.

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The Anti-streptococcal Action of Iodinine. Naphthaquinones and Anthraquinones as its Main Natural Antagonists

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Chromobacterium iodinum was isolated and so named owing to the purple, bronze-glinting pigment which covers its colonies on suitable solid media [Davis, 1939]. This pigment, here termed 'iodinin', was found to inhibit the growth of certain other bacteria [McIlwain, 1941a]; and such may be its natural function. The organisms so inhibited include pathogens, and the mechanism of the inhibition has now been studied, since the compound is highly active and an understanding of such processes is of potential chemotherapeutic value.

Iodinine (I) is the di-*N*-oxide of a dihydroxyphenazine. The positions of the hydroxyl groups