CXXIII. VITAMIN C IN THE ORANGE AND THE GRAPE FRUIT.

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INTRODUCTION.

THE experiments described in this report are a part of a general investigation concerned with the bearing of the antiscorbutic factor on the biological activity of the plant. They have been carried out in a similar way to those made in the study of this vitamin in the apple [Bracewell, Hoyle and Zilva, 1930; Bracewell, Kidd, West and Zilva, 1931; Bracewell, Wallace and Zilva, 1931], with the aim of correlating the antiscorbutic activity of the fruit with its environment.

In this communication the influence of the season on the potency of the orange and of season and variety on that of the grape fruit has been investigated. It is evident that carefully controlled material which could be conveniently transported to meet the requirements of the enquiry was necessary and with this end in view Palestine was chosen as the source of the orange and British Honduras and South Africa as those of the grape fruit.

Concordant results were obtained during two consecutive seasons and indications are available that the time of picking has no influence on the activity of the juice of the orange or of the grape fruit, and in the case of the latter no very significant difference was observed in the potency of the juice of the several varieties investigated. There is some evidence, however, that those fruits, which yield a diminished amount of juice with the progress of the season and on storage, also yield a correspondingly smaller amount of vitamin since there is no change in the minimum protective dose of the juice. This would suggest that the antiscorbutic factor or one or more of the substances which contribute to its stability [Zilva, 1927, 1928, 1929, 1930] are utilised in the process of ageing of the fruit.

TECHNIQUE.

The method of assay of vitamin C was the same as that employed in the previous investigation [Bracewell, Hoyle and Zilva, 1930]. The test was continued in most cases for 90 days. A prolonged test was important in these experiments since any serious deterioration in the antiscorbutic activity of a

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batch of fruit during a short period of storage would in all probability have been reflected in the weight curves.

THE ANTISCORBUTIC ACTIVITY OF THE JAFFA (SHAMUTI) ORANGE.

Oranges are exported from Palestine from November to April inclusive; the bulk of the fruit is normally shipped in December, January, February and March. It was therefore desirable to examine samples collected at the beginning, middle and end of the season. This was done in the first experiment in 1928/29. During the following season material from the beginning and from the end of the season only was tested for confirmatory purposes.

Nine adjacent trees, planted at 5.5 metres distance and bearing 27 cases of fruit, in a grove situated 3 miles north of Jaffa, were set aside for the experiment. Fruit was picked from all the nine trees at the different periods of the season. The trees, about 25 years old, were healthy and were budded on bitter orange stock (*Citrus bigaradia*) in their second year. They were growing on fairly heavy loamy soil. The manurial treatment was as follows.

To each tree was added:

100 kg.	old horse manure	in October, 1927.
1.125 „	sulphate of ammonia	in March, 1928.
„ „ „	„ „	June, 1928.
„ „ „	„ „	August, 1928.
100 „	old horse manure	in October, 1928.

The same group of trees was used for the 1928/29 and 1929/30 experiments. Table I gives the dates of picking *etc.* In the 1929/30 season the oranges were

Table I.

Batch	Picked	Beginning of biological test
V 1, 1928	Early November	11. xii. 28
V 4, 1929	Early January	5. ii. 29
V 9, 1929	Early March	15. iv. 29
V 1, 1929	Early December	2. i. 30
V 9, 1930	Early March	2. iv. 30

exceptionally late in maturing and consequently the first batch was picked about 3 weeks later than the corresponding batch of the previous year.

The tests for vitamin C were begun immediately on the arrival of the fruit. The oranges were stored at a temperature of about 15° during the three months' duration of the test. The juice was expressed daily before dosing and the quantity yielded by each fruit noted.

The chemical constitution of orange juice changes when the fruit is held on the tree and when kept in storage. During the process of ripening the soluble solids content of the juice increases, especially during the last 2 or 3 weeks [Copeman, 1931]. There are also a gradual increase of total sugar, sucrose and reducing sugars increasing at about the same rate, and a decrease of acidity as the oranges mature [Collinson, 1913].

Circumstances did not permit in this investigation of many chemical analyses being made, but the changes observed were broadly in agreement with those previously recorded by workers who had studied this aspect of the subject in greater detail.

Table II gives a 10-day average of the yield of juice per orange of the

Table II. *Daily average yield of juice per orange. The duration of the test is divided into 10-day periods.*

Period	V 1, 1928 cc.	V 4, 1929 cc.	V 9, 1929 cc.	V 1, 1929 cc.	V 9, 1930. A cc.	V 9, 1930. B cc.
1	67.60	65.70	42.46*	66.66	53.33	63.33
2	65.40	57.24*	50.75	69.00	48.50	65.50
3	63.70	68.29*	43.78	67.25	50.00	70.00
4	79.10*	60.43	41.50	61.30*	45.00*	60.00*
5	58.88*	54.62	41.11	65.33	53.01	58.33*
6	63.55	62.17	35.50	58.00	35.00	51.66
7	43.14	61.50	32.11	50.00	—	51.33
8	43.90	58.87	20.52	45.10	—	48.30

* Denotes period during which the juice was analysed. Results are given in Table III.

various batches as the tests proceeded. It is seen that the yield decreases as the test of each batch proceeds. It decreases further as the season advances. The last batch (V 9) of the 1929/30 season arrived in two different containers, A and B, and the oranges from each box were removed on alternate days for the tests so as to obtain an average result. It will be seen, however, that there was a marked difference in yield of juice by the fruit of the two consignments,

Table III.

Batch	Date of estimation	Total solids g./100 cc.	Acid in g. Citric acid/100 cc.	Maturity ratio	p _H of juice
V 1, 1928 Early November	12. i. 29	9.49	1.35	7.1	3.06
		8.72	1.29	6.8	3.11
		8.11	0.68	12.0	3.62
	25. i. 29	9.39	1.32	7.2	3.13
		9.43	1.27	7.4	3.23
V 4, 1929 Early January	19. ii. 29	10.93	0.94	11.6	3.29
		12.00	0.98	12.3	3.35
		9.86	1.37	7.2	3.23
	6. iii. 29	9.32	0.94	10.0	3.33
		9.83	1.10	8.9	3.23
V 9, 1929 Early March	15. iv. 29	12.50	0.73	17.0	3.75
		11.89	0.77	15.4	3.64
		12.81	0.46	27.7	3.95
	18. iv. 29	10.40	0.62	16.9	3.72
		11.55	0.33	35.4	4.20
V 1, 1929 Early December	12. ii. 30	10.42	0.79	13.15	3.02
		8.70	0.93	9.34	2.87
		9.02	0.79	11.58	2.99
	13. v. 30	10.57	1.17	9.06	3.45
		11.24	0.85	13.32	3.60
B 1930 Early March	9. v. 30	10.21	0.56	15.66	3.90
		11.89	0.79	15.02	3.72
	18. v. 30	10.61	0.95	11.23	3.48
		10.76	1.02	10.50	3.72

although they were picked at the same time and in the same grove. The average yield of the two is lower than that from the first batch, V 1, of this season.

Table III sets out the analytical data of the various batches. Unfortunately, as will be seen from Table II, the analyses could not be carried out in corresponding periods of the testing time. Nevertheless, they give a fair survey of the changes that took place in the composition of the juice with the progress of the season. It will be seen that in the 1928/29 season there was an increase in the soluble solids as the oranges remained longer on the tree. The acid content and the hydrogen ion concentration decreased. The maturity ratio (ratio of soluble solids to acidity calculated as citric acid) rose in consequence as the season advanced. The figures for the season 1929/30 did not show quite the same trend, but this is possibly due to the abnormality of the season.

The vitamin C assays (Table IV) do not reveal any marked difference in

Table IV. *Antiscorbutic potency of juice from Jaffa oranges.*

Dose cc.	V 1, 1928 Picked early November	V 4, 1929 Picked early January	V 9, 1929 Picked early March	V 1, 1929 Picked early December	V 9, 1930 Picked early March
$\frac{1}{2}$	No protection	No protection	?	+	+
1	+++	+++	+++	+++	+++
$1\frac{1}{2}$	Not tested	++++	++++	++++	++++
2	++++	++++	++++	Not tested	Not tested
3	++++	Not tested	Not tested	++++	++++

potency within the limits of the gradation of the tested doses. There was possibly a slightly higher potency in the juices of V 9, 1929 and V 1 and V 9, 1930. This disparity is, however, of such a low order that it cannot be considered significant. The appearance of the weight curves was normal and the *post mortem* findings were consistent, which suggests that a significant loss in the antiscorbutic potency was not incurred during at least the first two months of the test.

THE ANTISCORBUTIC ACTIVITY OF GRAPE FRUIT FROM BRITISH HONDURAS.

The export season in British Honduras lasts from October to the beginning of April, and samples were examined which were collected at the beginning, the middle and the end of the season during the first year and at the beginning and at the end of the season during the second year. Two varieties, namely Duncan and Marsh, were investigated. Owing to a misunderstanding the two varieties were mixed in the first season's tests. The following year however, each variety was tested separately in the autumn in order to ascertain whether they differed in potency.

The fruit was collected from two healthy trees from a grove in British Honduras situated 14 miles from the sea coast on the lower slope of a hill at an altitude of about 100 ft. above sea level. The trees were imported from the United States and were planted in the present grove in 1914. The age of

the trees and nature of the stock is not known. The tree from which the Duncan variety was picked is grown in 18 in. of reddish-brown, alluvial silt grading to a dry, dense bright red micaceous clay subsoil, p_H 6.9. The Marsh variety is grown on 14 in. of reddish brown, alluvial silt grading to a reddish yellow, gritty and micaceous dry clay subsoil, p_H 6.9. Grass grows throughout the grove which is grazed by mules. Grazing has caused the surface to become somewhat packed. The trees were sprayed with Bordeaux sulpho-citrol four times a year. 15 lb. per tree of a manurial mixture containing 10 % of phosphoric acid, 6 % nitrogen and 7 % of soluble potash was forked in during March and April.

The vitamin tests were begun immediately on the arrival of the fruit, which was stored at about 15°. The juice was expressed daily, but owing to the size of the fruit only a part of it was used. The remainder was kept in cold store at 0° until the fruit was finished, usually 3 days. Table V gives dates of picking and the beginning of the tests.

Table V.

Batch	Picked	Beginning of biological test
G 1, 1928. Marsh and Duncan	Early December	14. i. 29
G 3, 1929. Marsh and Duncan	Early January	20. iii. 29
G 4, 1929. Marsh and Duncan	Early March	10. iv. 29
G 1, 1929. Marsh	Early October	12. xi. 29
G 1, 1929. Duncan	Early October	13. xi. 29
G 4, 1930. Marsh	Early April	13. v. 30

The changes which take place in the chemical constitution of the grape fruit juice are briefly the following. Collinson [1913] found a decrease in acidity and an increase in the sugar content as the season advanced. After the fruit has matured the sucrose is gradually hydrolysed to reducing sugar. Zoller [1918] observed a decrease of acid content during storage and an increase of sugar after the removal of the fruit from the tree. According to Hawkins and Magness [1920] the acid content decreases and there is an apparent increase in sugar during cold storage. The dry matter increases during storage. There is a shrinkage from 5-8 % in cold storage to 25 % in warm ventilated storage. Hawkins [1921] found further that in warm storage there is a marked increase in the percentage of the acid calculated on the wet weight of the pulp, which is entirely due to loss of water. There is also possibly a slight decrease in the sugar content. In cold storage the acidity decreases markedly after 4 months whilst there is little change in the sugar content.

Table VI presents the analytical data obtained from the fruit used in this investigation. No very marked difference between the various periods can be discerned from these few data. The figures, however, suggest that the soluble solids and the acid content of the Duncan variety of 1929/30 season were lower than those of the Marsh variety of the same season.

The antiscorbutic potency of the grape fruit is summarised in Table VII. The general evidence points to the fact that this fruit, too, does not vary

Table VI.

Batch	Date of estimation	Total solids g./100 cc.	Acid in g. citric acid/100 cc.	p _H of juice
G 1, 1928	28. ii. 29	9.76	1.03	3.16
Marsh and Duncan		10.43	0.58	3.65
Early December	4. iii. 29	11.45	0.74	3.23
		10.83	0.76	3.30
G 3, 1929	7. iii. 29	10.53	0.88	3.32
Marsh and Duncan		12.97	1.18	3.18
Early January	23. iii. 29	10.12	0.75	3.43
		10.27	0.66	3.51
G 4, 1929	10. iv. 29	9.89	0.87	3.35
Marsh and Duncan		9.97	0.62	3.62
Early March	16. iv. 29	11.34	0.73	3.48
		11.61	0.76	3.44
G 1, 1929	8. i. 30	10.24	1.17	3.29
Marsh		10.56	1.10	3.20
Early October				
G 1, 1929	8. i. 30	8.90	0.89	3.16
Duncan		8.81	0.69	3.31
Early October				
G 4, 1930	16. vi. 30	10.08	0.95	3.25
Marsh		10.45	1.00	3.38
Early April		10.26	1.09	3.29

Table VII. *Antiscorbutic potency of juice from grape fruit. British Honduras.*

	G 1, 1928 Marsh and Duncan	G 3, 1929 Marsh and Duncan	G 4, 1929 Marsh and Duncan	G 1, 1929 Marsh	G 1, 1929 Duncan	G 4, 1930 Marsh
Dose cc.	Picked early December	Picked early January	Picked early March	Picked early October	Picked early October	Picked early April
$\frac{1}{2}$	++	?	?	++	+++	++
1	++++?	+++	+++	++++	++++	++++?
$1\frac{1}{2}$	Not tested	++++	++++	++++	++++	++++
2	++++	++++	++++	Not tested	Not tested	Not tested
3	++++	Not tested	Not tested	Not tested	Not tested	++++

within the limits of the dosage used in these tests, with the time of season. Nor do the weight curves reveal a very marked loss in activity during the first 2 months of the test period. The results call, however, for some comment. The mixed juices of the Marsh and Duncan of December 1928 showed a somewhat higher vitamin content than did those of January and of March 1929. The difference in activity is of an order which falls outside the limits of the experimental error. It is difficult, however, to accept the view that this falling off in activity was due to the longer sojourn of the fruit on the tree, since no significant difference was observed between the activity of the Marsh variety picked in October and that of this fruit picked in April of the following season.

A somewhat marked falling off in weight of the guinea-pigs on the 1 cc. dose of the Marsh picked in April 1930 was observed towards the end of the test. This could hardly be due to the deterioration of the activity of the fruit during the test period, since the manifestation of scorbutic symptoms should

have preceded the decline in weight if it were due to scurvy. This did not occur except in one animal. Nor did the *post mortem* examination of the animals suggest that the loss in weight was due to scurvy.

It must be noted that the Duncan variety, in spite of lower total solids, showed a somewhat higher vitamin content than the Marsh variety.

THE ANTISCORBUTIC ACTIVITY OF GRAPE FRUIT FROM SOUTH AFRICA.

These experiments were instituted in order to ascertain whether there is a difference in the antiscorbutic potency of the same variety (Marsh) emanating from a different environment. An additional variety (Florida) was also assayed.

The South African grape fruit season lasts from the middle of May to the middle of August. The two varieties were received early in September.

Table VIII. *Antiscorbutic potency of juice from grape fruit.*
South Africa.

Dose (cc.)	1930. Marsh	1930. Florida
$\frac{1}{2}$	No protection	+
1	++++	++++
$1\frac{1}{2}$	++++	++++
3	++++	++++

The Marsh variety was grown at Simondium, Western Cape, on Karroo silt. The trees were 12 years old, healthy and budded on Rough Cape Lemons. They were planted in the grove in 1918. They were previously bad with scale, but are fairly clear now and were not sprayed. The soil was ploughed annually and has been irrigated six times annually since March 1928. Previous to this, owing to drought, irrigation was erratic, the trees going sometimes for as long as six months without water. The irrigation was followed by cultivation. They have been fertilised each autumn with kraal manure since 1926.

The Florida variety was grown in the Eastern Cape Province on Karroo silt. The trees were 8 years old, healthy and budded on Rough Cape Lemons. They were planted in 1922 and were not sprayed. The method of irrigation, cultivation and manuring was the same as for the Marsh variety.

As will be seen from Table IX, these batches of grape fruit had a higher

Table IX.

Batch	Date of estimation	Total solids g./100 cc.	Acid in g. citric acid/100 cc.	p_H of juice
1930. Marsh	2. x. 30	10.31	1.44	2.71
	14. x. 30	9.67	1.46	2.80
	17. x. 30	10.18	1.78	2.83
1930. Florida	2. x. 30	8.69	1.69	2.83
	14. x. 30	8.51	1.78	2.87
	17. x. 30	8.26	1.76	2.71

acid content and lower p_H value than the British Honduras varieties. The Florida variety showed a total solids content similar to that of the Duncan variety from British Honduras. The vitamin content (Table VIII) was of the

same order as that of the corresponding British Honduras Marsh variety. It is true that the South African Marsh variety shows slightly lower activity than the one of British Honduras origin, but the difference is too small to be considered significant under the circumstances.

CONCLUSIONS.

These systematic observations on the constant antiscorbutic activity of the orange and grape fruit during the entire season and on the stability of the vitamin in the intact fruit are consistent with the scattered observations made by various workers in this connection. The vitamin C activity of the orange, lemon and grape fruit has very seldom been found to vary to any great extent and evidence is also available concerning its stability. Thus Davey [1921] observed that 5 months of cold storage did not appreciably impair the vitamin C content of the orange. The activity of the orange, as we have seen from the foregoing experiments, like that of the grape fruit, does not seem to fluctuate markedly with the variety. The minimum dose necessary to protect the guinea pig from scurvy, as found in this investigation, is about the same as that recorded by Delf [1921] and Davey [1921] for Denia and Navel oranges. Taking all the available information into consideration one may safely assume that oranges and grape fruit bought casually in the open market under normal conditions ought to possess the full antiscorbutic value characteristic of these fruits.

A point of academic interest which requires further investigation is whether the total amount of vitamin of the Citrus fruits diminishes as they age on the trees or in storage. The yield of juice of the Jaffa orange diminished with the progress of the season when the fruit was left on the tree and during the 3 months' storage in the course of carrying out the test. The antiscorbutic potency of the juice on the other hand did not show an increase. The difference in the yield of the orange juice between the first and last pickings of the 1928/29 season ranged from 25 to 53 % for the corresponding periods, the average being 35 % (Table II). A similar increase in the potency in the juice should have been reflected in the test. A closer spacing of the doses would most probably establish definitely whether or not such an increase takes place.

The small disparity in the vitamin C content of the orange, grape fruit, lemon and sweet lime (*Citrus limetta*) as contrasted with the West Indies lime (*Citrus medica* var. *acida*), which possesses a definitely lower antiscorbutic activity than the aforementioned Citrus fruits, presents an analogy to the variation in the antiscorbutic activity amongst the apple varieties. In the apples, the antiscorbutic activity of which is on a lower plane than that of the Citrus fruits, the Bramley's Seedling, and, according to evidence which is now forthcoming, also some other varieties are markedly more potent than another category of apples such as Cox's Orange Pippin, Worcester Pearmain *etc.*, which differ amongst themselves comparatively little [Bracewell, Hoyle

and Zilva, 1930]. This sharp division in antiscorbutic activity amongst closely related fruits is rather striking and calls for further attention.

SUMMARY.

1. The antiscorbutic activity has been determined of (a) the Jaffa orange picked from the same trees at different times of the season during two consecutive years, (b) of the Marsh and Duncan varieties (British Honduras) of grape fruit also picked at different times of the season during two consecutive years and (c) the Marsh and Florida varieties (South Africa) of grape fruit picked at the end of the season.

2. Within the limits of the doses tested and of the accuracy of the method employed, the vitamin content of the juice is the same whether the orange or grape fruit is picked at the beginning or at the end of the season.

3. There is no marked loss in the vitamin content when the fruit is stored under ordinary conditions at about 15° for about 2 months.

4. The conditions of cultivation, origin of stock, age of tree, soil *etc.* do not affect the vitamin C content of the fruit.

5. The Marsh variety of grape fruit whether of British Honduras or of South African origin, is of approximately the same potency.

6. The Duncan variety of grape fruit (one test only) was found to be slightly more active than the other two varieties of grape fruit tested.

7. The vitamin C content of the grape fruit was rather higher than that of the Jaffa orange.

8. There is no correlation between the soluble solids, acid content, p_H of the juice and its antiscorbutic potency.

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