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RESULTS AND DISCUSSION

As stated earlier studies were carried out to detect the glycolytic enzymes and their intermediates as well as enzymes involved in the synthesis and oxidation of ascorbic acid and their intermediates in the edible portion of citrus fruit (Citrus acida of 1.4-1.6 cm diameter).

The dependence of enzyme activity on enzyme and substrate concentrations, period of incubation and pH for different enzymes are shown in Tables 3-11. The activity of each enzyme increased linearly with enzyme and substrate concentration and period of incubation within a certain range.

In the case of the enzyme system synthesizing ascorbic acid the omission of the substrate did not affect the synthesis. It was thought that this might have been due to the presence of endogenous substrate in the enzyme preparation. The enzyme was therefore dialysed overnight against distilled water and tried for its capacity to effect the synthesis. It was found that the dialysed preparation had lost its activity. Further the activity of even the undialysed enzyme preparation was found to be very low. This suggests that the assay system used is not perhaps the most appropriate. The data obtained on this enzyme therefore should be discussed with reservation till we get a better system to show the effective

Table 3. Effect of enzyme concentration, period of incubation, glucose concentration and pH on hexokinase activity in the fruit tissues of Citrus acida.* +

Variable	NADPH ₂ formed (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.00	0.000
0.05	0.012
0.10	0.028
0.15	0.040
0.20	0.051
0.25	0.054
0.30	0.054
<u>Period of incubation (minutes)</u>	
0	0.000
1	0.008
2	0.019
3	0.028
4	0.041
5	0.051
6	0.056
7	0.056
<u>Micromoles of glucose</u>	
0.0	0.000
0.5	0.015
1.0	0.033
1.5	0.051
2.0	0.058
pH (phosphate buffer was used upto pH 7.0 and tris-HCl buffer was used for pH 7.0-9.0)	
Phosphate : 6.0	0.032
buffer : 7.0	0.042
: 7.0	0.044
Tris-HCl : 7.5	0.051
buffer : 8.0	0.051
: 9.0	0.047

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ A 33% tris homogenate was prepared from edible portion of the fruit (1.4-1.6 cm diameter), centrifuged at 6500 x g and the supernatant used as enzyme source.

**Protein content, 1.4 mg/ml.

Table 4. Effect of enzyme concentration, period of incubation, glucose-6-phosphate concentration and pH on phosphohexose isomerase activity in the fruit tissues of Citrus acida.*+

Variable	Fructose-6-phosphate formed (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.000	0.00
0.025	0.18
0.050	0.42
0.075	0.65
0.100	0.88
0.125	0.92
0.150	0.95
<u>Period of incubation (minutes)</u>	
0	0.00
10	0.30
20	0.57
30	0.88
40	0.96
50	1.12
<u>Micromoles of glucose-6-phosphate</u>	
0	0.00
1	0.15
2	0.33
3	0.50
4	0.66
5	0.88
6	0.93
pH (phosphate buffer was used upto pH 7.0 and tris-HCl buffer for pH 7.0-9.0 and glycine-NaOH buffer for pH 9-10)	
Phosphate buffer	6.0 0.65
	7.0 0.80
Tris-HCl buffer	7.0 0.81
	8.0 0.82
	9.0 0.88
Glycine-NaOH buffer	9.0 0.86
	10.0 0.76

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ A 33% tris homogenate, prepared from edible portion of the fruit (1.4-1.6 cm diameter), was used.

**Protein content, 1.5 mg/ml.

Table 5. Effect of enzyme concentration, period of incubation, fructose-6-phosphate concentration and pH on phosphofructokinase activity in the fruit tissues of Citrus acida.*+

Variable	Fructose diphosphate formed (micromoles)
<u>Amount (ml) of enzyme**preparation</u>	
0.2	0.20
0.4	0.38
0.5	0.56
0.6	0.60
0.7	0.64
<u>Period of incubation (minutes)</u>	
0	0.00
10	0.17
20	0.36
30	0.56
40	0.64
50	0.64
<u>Micromoles of fructose-6-phosphate</u>	
0	0.00
2	0.22
4	0.43
5	0.56
6	0.56
8	0.60
pH (tris-HCl buffer was used for pH 7.0-9.0 and glycine NaOH buffer for pH 9.0-10.0)	
Tris-HCl : 7.0	0.38
buffer : 8.0	0.45
: 9.0	0.56
Glycine- : 9.0	0.56
NaOH : 10.0	0.48
buffer :	

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ Crude 33% phosphate homogenate, prepared from edible portion of the fruit (1.4-1.6 cm diameter), was used.

**Protein content, 1.6 mg/ml.

Table 6. Effect of enzyme concentration, period of incubation, fructose, 1,6-diphosphate concentration and pH on fructose diphosphate aldolase activity in the fruit tissues of Citrus acida.*+

Variable	Triosephosphates formed (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.000	0.00
0.025	0.17
0.050	0.36
0.075	0.55
0.100	0.70
0.125	0.78
<u>Period of incubation (minutes)</u>	
0	0.00
10	0.25
20	0.48
30	0.70
40	0.81
50	0.88
<u>Micromoles of fructose-1,6-diphosphate</u>	
0.00	0.00
0.50	0.15
1.00	0.32
1.50	0.51
2.00	0.70
2.50	0.78
<u>pH (tris-HCl buffer used)</u>	
7.0	0.59
7.5	0.63
8.0	0.70
8.5	0.66
9.0	0.62

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+A 33% tris homogenate, prepared from edible portion of the fruit (1.4-1.6 cm diameter), was used.

**Protein content, 1.5 mg/ml.

Table 7. Effect of enzyme concentration, period of incubation, glyceraldehyde-3-phosphate concentration and pH on glyceraldehyde-3-phosphate dehydrogenase activity in the fruit tissues of Citrus acida.*+

Variable	NADH ₂ formed (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.000	0.000
0.025	0.012
0.050	0.025
0.075	0.037
0.100	0.050
0.125	0.056
<u>Period of incubation (minutes)</u>	
0	0.000
1	0.017
2	0.035
3	0.050
4	0.058
5	0.058
<u>Micromoles of glyceraldehyde-3-phosphate</u>	
0.00	0.000
0.25	0.014
0.50	0.026
0.75	0.039
1.00	0.050
1.25	0.050
<u>pH (tris-HCl buffer used)</u>	
7.0	0.032
7.5	0.036
8.0	0.042
8.5	0.050
9.0	0.050

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ A 33% tris homogenate was prepared from edible portion of the fruit (1.4-1.6 cm diameter), centrifuged at 6500 x g and the supernatant used as enzyme source.

**Protein content, 1.4 mg/ml.

Table 8. Effect of enzyme concentration, period of incubation, pyruvate concentration and pH on lactate dehydrogenase activity in the fruit tissues of Citrus acida.*+

Variable	NADH ₂ oxidized (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.000	0.000
0.025	0.012
0.050	0.025
0.075	0.038
0.100	0.050
0.125	0.054
<u>Period of incubation (minutes)</u>	
0	0.000
1	0.008
2	0.018
3	0.030
4	0.041
5	0.050
6	0.055
7	0.055
<u>Micromoles of sodium pyruvate</u>	
0.00	0.000
0.25	0.013
0.50	0.024
0.75	0.037
1.00	0.050
1.25	0.056
<u>pH (phosphate buffer used)</u>	
6.0	0.032
6.5	0.043
7.0	0.050
7.5	0.050
8.0	0.047

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+A 33% tris homogenate was prepared from edible portion of the fruit (1.4-1.6 cm diameter), centrifuged at 6500 x g and the supernatant used as enzyme source.

**Protein content, 1.4 mg/ml.

Table 9. Effect of enzyme concentration, period of incubation, glucose-1-phosphate concentration and pH on phosphoglucomutase activity in the fruit tissues of Citrus acida.*+

Variable	Glucose-1-phosphate disappeared (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.00	0.00
0.05	0.08
0.10	0.15
0.15	0.23
0.20	0.30
0.25	0.34
<u>Period of incubation (minutes)</u>	
0	0.00
5	0.09
10	0.17
15	0.30
20	0.35
25	0.39
<u>Micromoles of glucose-1-phosphate</u>	
0	0.00
2	0.12
4	0.22
5	0.30
6	0.32
8	0.32
<u>pH (tris-HCl buffer used)</u>	
7.0	0.22
7.5	0.30
8.0	0.26
8.5	0.24
9.0	0.24

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ A 33% tris homogenate, prepared from edible portion of the fruit (1.4-1.6 cm diameter), was used.

**Protein content, 1.5 mg/ml.

Table 10. Effect of enzyme concentration, period of incubation and pH on ascorbic acid synthesis in the fruit tissues of Citrus acida.*+

Variable	Ascorbic acid formed (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.00	0.00
0.50	0.38
1.00	0.80
1.50	1.30
2.00	1.60
2.50	1.60
<u>Period of incubation (minutes)</u>	
0	0.00
30	0.35
60	0.80
90	1.30
120	1.60
150	1.90
180	2.10
<u>pH (phosphate buffer was used)</u>	
6.0	1.1
7.0	1.6
7.5	1.4
8.0	1.4

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ A 33% sucrose homogenate was prepared from edible portion of the fruit (1.4-1.6 cm diameter), centrifuged at 500 x g and the supernatant used as enzyme source.

**Protein content, 1.6 mg/ml.

Table 11. Effect of enzyme concentration, period of incubation, ascorbic acid concentration and pH on ascorbic acid oxidase activity in the fruit tissues of Citrus acida.*+

Variable	Oxygen utilized (micromoles)
<u>Amount (ml) of enzyme** preparation</u>	
0.0	0.0
0.5	2.6
1.0	5.2
1.5	7.7
2.0	10.0
2.5	10.6
<u>Period of incubation (minutes)</u>	
0	0.0
15	1.9
30	4.8
45	7.4
60	10.0
75	11.2
90	12.2
<u>Micromoles of ascorbic acid</u>	
0	0.4
20	3.0
40	6.5
60	10.0
80	12.0
100	12.8
<u>pH (phosphate buffer used)</u>	
6.0	7.8
6.5	8.6
7.0	10.0
7.5	9.2
8.0	6.0

* Assay system was the same as that given in Table 2 except for the variables mentioned.

+ Crude 25% phosphate homogenate, prepared from edible portion of the fruit (1.4-1.6 cm diameter), was used.

**Protein content, 1.1 mg/ml.

synthesis. The optimum conditions used on the basis of the above for assay of different enzymes are given in Table 12.

The pH optima found in the present studies are compared with those for the enzymes derived from other sources in Table 13.

With regard to optimum pH, hexokinase of Citrus acida resembles that of excised roots of Phaseolus mungo L. (Parekh, 1965). Phosphohexose isomerase resembles that of etiolated corn seedlings (Black and Humphreys, 1962) and muscle (Slein, 1955). Phosphofructokinase has a higher optimal pH than that of rabbit brain (Buell et al., 1958). Fructose diphosphate aldolase and glyceraldehyde-3-phosphate dehydrogenase resemble that of rabbit muscle (Neilands and Stumpf, 1955). Lactate dehydrogenase has a lower optimum pH than that of rabbit muscle (Kornberg, 1955) as well as that of excised roots of Phaseolus mungo L. (Parekh, 1965). These remarks are subject to the reservation that the preparations used in the present experiment are crude homogenates or cell free extracts.

The data given in Table 12 show that fruit tissues of Citrus acida possess the key enzymes of glycolysis. This along with the demonstration of the TCA cycle enzymes (Ramakrishnan and Varma, 1959) suggest the capacity of the fruit tissues to convert sugar into citric acid. Similarly

Table 12. Activities of enzymes of glycolytic cycle and ascorbic acid synthesizing enzyme and ascorbic acid oxidase in the fruit tissues of Citrus acida (1.4-1.6 cm diameter).

Enzyme	Amount required in the assay system for optimum activity		Optimum period of incubation (minutes)	Optimum pH	Specific activity under the conditions specified
	Enzyme (mg)	Substrate (micromoles)			
Hexokinase	0.28	1.5	5	7.5	2.2
Phosphohexose isomerase	0.15	5.0	30	9.0	11.7
Phosphofructokinase	0.80	5.0	30	9.0	1.4
Fructosediphosphate aldolase	0.15	2.0	30	8.0	9.3
Glyceraldehyde-3-phosphate dehydrogenase	0.14	1.0	3	8.5	7.1
Lactate dehydrogenase	0.14	1.0	5	7.0	4.3
Phosphoglucumutase	0.30	5.0	15	7.5	4.0
Ascorbic acid synthase	3.20	-	120	7.0	0.3
Ascorbic acid oxidase	2.20	60.0	60	7.0	4.5

Table 13. pH optima for the glycolytic enzymes obtained from different sources.

Enzyme	Optimum pH			reference
	Citrus acids (present study)	material used	Other sources pH	
Hexokinase	7.5	Excised roots of <u>Phaseolus mungo</u> L.	7.5 Parekh (1965)	
		Excised roots of <u>Trigonella foenum græcum</u> L.	8.0 Antony et al (1965)	
Phosphohexose isomerase	9.0	Etiolated corn seedlings Muscle	9.0 Black and Humphreys (1962)	
			9.0 Slein (1955)	
Phosphofructo- kinase	9.0	<u>Phaseolus radiatus</u> Rabbit brain	7.8 Ramasarma and Giri (1956)	
			8.0 Buell et al (1958)	
Fructose diphos- phate aldolase	8.0	Rabbit muscle	7.5- 8.5 Neillands and Stumpf (1955)	
		Rat tissues	8.6 Sibley and Lehninger (1949)	
		Yeast	7.4 Christian (1955)	
Glyceraldehyde 3-phosphate dehydrogenase	8.5	Yeast	8.3- 8.5 Krebs (1955)	
		Rabbit muscle	8.5 Neillands and Stumpf (1955)	
Lactate dehydro- genase	7.0	Rabbit muscle	7.4 Kornberg (1955)	
		Excised roots of <u>Phaseolus mungo</u> L.	7.5 Parekh (1965)	



the presence of the hexokinase, phosphoglucomutase, ascorbic acid synthesizing enzyme and ascorbic acid oxidase suggest the possible conversion of glucose to ascorbic acid and its break-down to dehydroascorbic acid.

Table 14 gives the data on the presence of total and free sugar, sugar phosphates, lactic acid, ascorbic acid and dehydroascorbic acid. Though the presence of these can not be interpreted without ambiguity, the presence of intermediates of glycolysis and substances involved in ascorbic acid metabolism along with that of the enzymes involved strongly suggests the metabolic capacity of fruit tissues to utilize sugar for the production of citric acid and ascorbic acid.

The next question which arises is the localization of these enzymes in different parts of the fruit at different stages of development.

The specific activity of hexokinase in different parts of the fruit during development is given in Table 15 and Fig. 5. The juice does not show any activity, the green skin shows the highest activity and the activity decreases in the order green skin, white skin, vesicles and septa. While the activity seems to reach a maximum between 1.4-1.6 cm diameter in the case of green skin, it continues to increase with the development of the fruit in the case of the white skin and vesicles.

Table 14. Chemical composition of the fruit tissues of Citrus acida (1.4-1.6 cm diameter).*
(Values expressed as micromoles $\times 10^5$ per 100g dry weight)

Constituent	Micromoles $\times 10^5$ per 100g dry weight
Total sugar	1.375 (1.25,1.50)
Free sugar	0.8 (0.7,0.9)
Glucose	0.0465 (0.042,0.051)
Glucose-1-phosphate	0.001 (0.001,0.001)
Glucose-6-phosphate	0.0018 (0.0017,0.0019)
Fructose-6-phosphate	0.0035 (0.003,0.004)
Fructose-1,6-diphosphate	0.0005 (0.0004,0.0006)
Lactic acid	0.027 (0.026,0.028)
Free ascorbic acid	0.012 (0.011,0.013)
Dehydroascorbic acid	0.006 (0.006,0.006)

* Mean of two trials. Individual values are given in the parentheses.

Table 15. Activity of hexokinase in different parts of the fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of hexokinase				
	Diameter of the fruit (cm)				
	0.8-1.0	1.4-1.6	2.4-2.6	4.0	
Green skin	4.0 (3.2,4.4,4.4)	10.0 (8.0,10.0,12.0)	6.8 (4.8,7.2,8.4)	6.8 (5.6,7.2,7.6)	
White skin	1.2 (0.8,0.8,2.0)	4.8 (3.6,5.2,5.6)	5.6 (4.4,5.6,6.8)	9.2 (7.6,9.6,10.4)	
Septa	0.4 (0.36,0.36,0.48)	0.8 (0.4,0.8,1.2)	3.2 (2.8,3.2,3.6)	2.0 (1.6,2.0,2.4)	
Vesicles	2.0 (1.6,2.0,2.4)	2.4 (1.6,2.0,3.6)	4.0 (3.6,3.6,4.8)	4.8 (3.6,4.8,6.0)	
Juice	N.D.	N.D.	N.D.	N.D.	

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Fig. 5 : Activity of hexokinase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

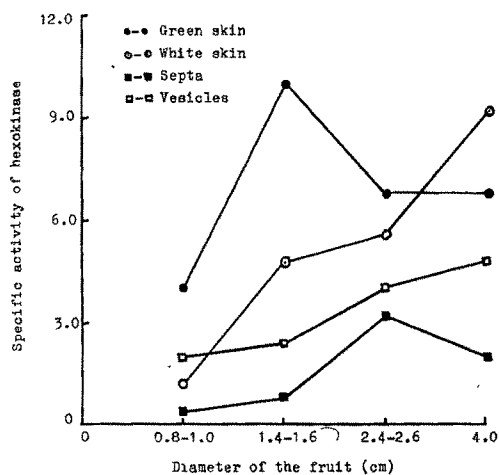


Fig. 6 : Activity of phospho-hexose isomerase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

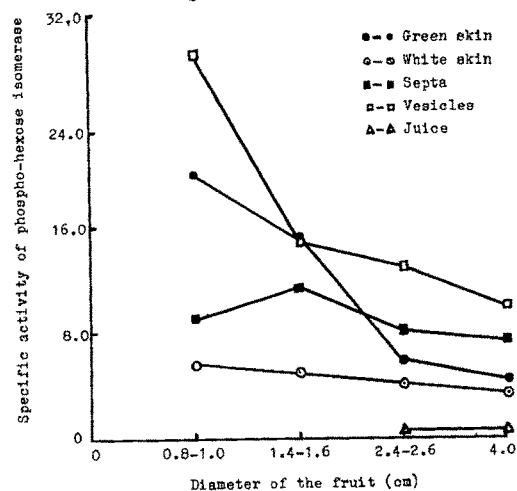


Fig. 7 : Activity of phospho-fructokinase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

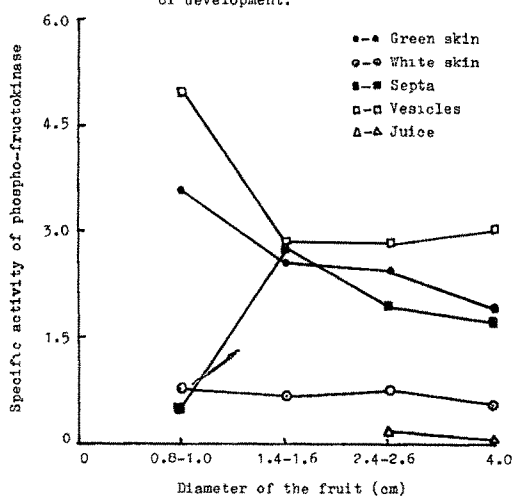
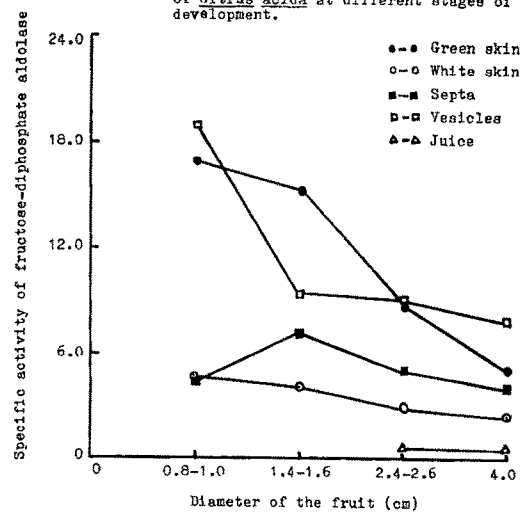


Fig. 8 : Activity of fructose-diphosphate aldolase in different parts of the fruit tissues of *Citrus acida* at different stages of development.



The specific activity of phosphohexose isomerase in different parts of the fruit during development is given in Table 16 and Fig. 6. The juice shows slight activity in the larger fruits but this may be due to enzyme contamination from other parts during the preparation of the juice. Regarding other parts the activity decreases in the order vesicles, green skin, septa and white skin. In all cases the activity decreases as the fruit develops and the maximum activity is seen in the young fruits.

The specific activity of phosphofructokinase in different parts of the fruit during development is given in Table 17 and Fig. 7. Again the juice shows practically no activity. The activity decreases in the order vesicles, green skin, septa and white skin. In the case of the vesicles the activity does not change with size after a diameter of 1 cm is reached while in the case of green skin and septa it decreases with the development of the fruit. In the case of the white skin it is the same at all stages of development. In the case of this enzyme also the young fruit shows maximum activity.

The specific activity of fructose diphosphate aldolase in different parts of the fruit during development is given in Table 18 and Fig. 8. Again the juice shows slight activity at later stages of development which may be due to

Table 16. Activity of phospho-hexoseisomerase in different parts of ^{the} fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of phospho-hexoseisomerase				
	Diameter of the fruit (cm)				
	0.8-1.0	1.4-1.6	2.4-2.6	4.0	
Green skin	20.0 (18.9, 19.9, 21.2)	15.3 (14.2, 15.0, 16.7)	6.2 (5.7, 6.2, 6.7)	4.5 (4.1, 4.5, 4.9)	
White skin	5.6 (4.5, 5.8, 6.5)	4.9 (4.1, 5.0, 5.6)	4.2 (3.8, 4.1, 4.7)	3.4 (3.1, 3.4, 3.7)	
Septa	9.3 (6.8, 9.5, 11.6)	11.5 (9.6, 11.3, 13.6)	8.2 (7.8, 8.3, 8.5)	7.4 (7.2, 7.2, 7.8)	
Vesicles	29.0 (27.2, 28.8, 31.0)	15.0 (14.0, 15.0, 16.0)	13.0 (12.0, 13.0, 14.0)	10.0 (9.7, 10.1, 10.2)	
Juice	N.D.	N.D.	0.7 (0.4, 0.7, 1.0)	0.7 (0.3, 0.6, 1.2)	

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Table 17: Activity of phospho-fructokinase in different parts of ^{the} fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of phospho-fructokinase			
	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	3.6 (2.9,3.4,4.5)	2.6 (1.9,2.4,3.4)	2.5 (2.1,2.4,3.0)	2.0 (1.8,2.0,2.2)
White skin	0.8 (0.6,0.8,1.0)	0.7 (0.6,0.7,0.8)	0.8 (0.6,0.8,1.0)	0.6 (0.4,0.6,0.8)
Septa	0.5 (0.3,0.4,0.8)	2.8 (1.8,2.9,3.7)	2.0 (1.7,2.0,2.3)	1.8 (1.6,1.7,2.1)
Vesicles	5.0 (4.2,4.7,6.1)	2.9 (1.9,2.9,3.9)	2.9 (2.3,2.8,3.6)	3.1 (2.8,3.2,3.3)
Juice	N.D.	N.D.	0.2 (0.1,0.2,0.3)	0.1 (0.03,0.07,0.2)

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Table 18. Activity of fructosediphosphate aldolase in different parts of ^{the} fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of fructosediphosphate aldolase				
	Diameter of the fruit (cm)				
	0.8-1.0	1.4-1.6	2.4-2.6	4.0	
Green skin	17.0 (14.2,17.6,19.2)	15.3 (12.8,14.5,18.6)	8.8 (7.6,8.6,10.2)	5.1 (4.8,5.0,5.5)	
White skin	4.6 (3.8,4.3,5.7)	4.1 (2.9,3.9,5.5)	2.9 (2.5,2.9,3.3)	2.4 (1.8,2.5,2.9)	
Septa	4.4 (3.7,3.8,5.7)	7.2 (6.7,7.2,7.7)	5.0 (4.6,4.9,5.5)	4.0 (3.5,3.7,4.8)	
Vesicles	19.0 (18.0,18.0,21.0)	9.4 (7.8,8.7,11.7)	9.0 (8.4,9.2,9.4)	7.8 (7.3,7.7,8.4)	
Juice	N.D.	N.D.	0.6 (0.5,0.5,0.8)	0.5 (0.2,0.6,0.7)	

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

enzyme contamination during the preparation of the extract. The vesicles and green skin show comparable activity and the septa shows more activity than the white skin. The activity generally decreases with the development of the fruit.

The specific activity of glyceraldehyde-3-phosphate dehydrogenase in different parts of the fruit during development is given in Table 19 and Fig. 9. In this case also the green skin and vesicles show comparable activity which is more than that in the septa. The white skin shows very low activity. The activity decreases with development in the green skin, septa and vesicles.

The specific activity of lactate dehydrogenase in different parts of the fruit during development is given in Table 20 and Fig. 10. While the juice does not show any activity, the activity in other parts decreases in the order vesicles, green skin, septa and white skin. In the case of the vesicles the activity remains almost constant after 1.0 cm diameter whereas in the case of septa it increases with the development.

To summarize, the juice is almost devoid of glycolytic enzymes. The vesicles show in general a higher activity of glycolytic enzymes than the green skin and septa. The white skin seems to show relatively less activity. The changes discussed above are summarized in Table 21.

Table 19. Activity of glyceraldehyde-3-P04 dehydrogenase in different parts of the fruit tissues of Citrus acid at different stages of development.

Part of the fruit	Specific activity of glyceraldehyde-3-P04 dehydrogenase			
	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	16.0 (13.4, 15.8, 18.8)	12.0 (11.2, 12.0, 12.8)	7.0 (6.3, 6.8, 7.9)	5.0 (4.7, 5.0, 5.3)
White skin	0.65 (0.6, 0.65, 0.7)	0.6 (0.5, 0.6, 0.7)	0.8 (0.7, 0.8, 0.9)	0.9 (0.8, 0.9, 1.0)
Septa	6.5 (6.0, 6.5, 7.0)	7.1 (6.8, 6.9, 7.6)	6.7 (6.3, 6.7, 7.1)	3.5 (3.2, 3.5, 3.8)
Vesicles	16.0 (14.5, 16.6, 16.9)	9.6 (8.0, 9.8, 11.0)	9.0 (7.8, 9.4, 9.8)	5.8 (4.6, 6.1, 6.7)
Juice	N.D.	N.D.	N.D.	N.D.

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Table 20. Activity of lactate dehydrogenase in different parts of ^{the} fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of lactate dehydrogenase			
	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	2.3 (1.5, 2.2, 3.2)	2.2 (1.7, 2.1, 2.8)	2.3 (1.8, 2.3, 2.8)	1.0 (0.7, 1.0, 1.3)
White skin	0.5 (0.4, 0.5, 0.6)	0.5 (0.4, 0.5, 0.6)	0.7 (0.6, 0.7, 0.8)	0.5 (0.4, 0.4, 0.7)
Septa	0.2 (0.1, 0.2, 0.3)	0.5 (0.4, 0.5, 0.6)	1.5 (0.9, 1.7, 1.9)	1.7 (1.2, 1.7, 2.2)
Vesicles	0.7 (0.6, 0.7, 0.8)	4.2 (3.5, 4.1, 5.0)	4.2 (3.9, 4.2, 4.5)	3.2 (2.6, 2.7, 4.3)
Juice	N.D.	N.D.	N.D.	N.D.

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Table 21. Glycolytic enzymes in the fruit tissues of Citrus acida.*

Enzyme	Part showing			Change with development of fruit
	maximum	minimum activity	intermediate	
Hexokinase	Green skin	Septa	White skin, vesicles	increases in white skin and vesicles and decreases in green skin
Phospho-hexose isomerase	Vesicles	White skin	Green skin, septa	decreases
Phosphofructokinase	Vesicles	White skin	Green skin, septa	decreases in the case of green skin and septa, no change in the case of vesicles and white skin
Fructose diphosphate aldolase	Vesicles, green skin	White skin	Septa	decreases
Glyceraldehyde-3-phosphate dehydrogenase	Vesicles, green skin	White skin	Septa	decreases in all the cases except white skin in which there is slight increase
Lactate dehydrogenase	Vesicles	White skin	Green skin, septa	In vesicles after 1 cm practically no change. In septa it increases. In white skin and green skin practically no change

* Juice is not included as it does not seem to possess the enzymes.

Fig. 9 : Activity of glyceraldehyde-3-P₀ dehydrogenase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

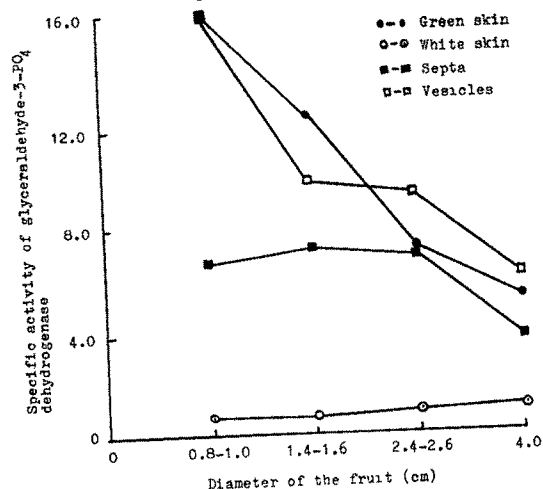


Fig. 10 : Activity of lactate dehydrogenase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

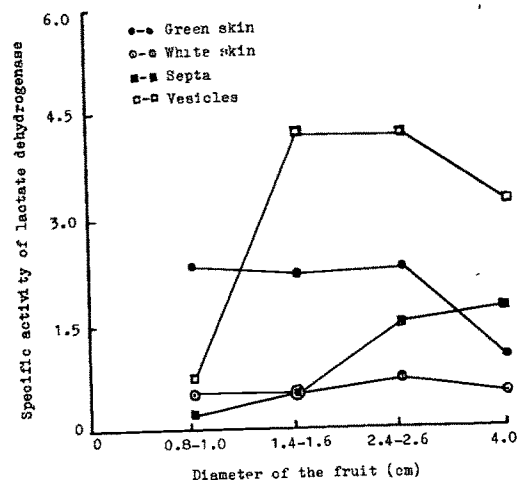


Fig. 11 : Activity of phospho-glucumutase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

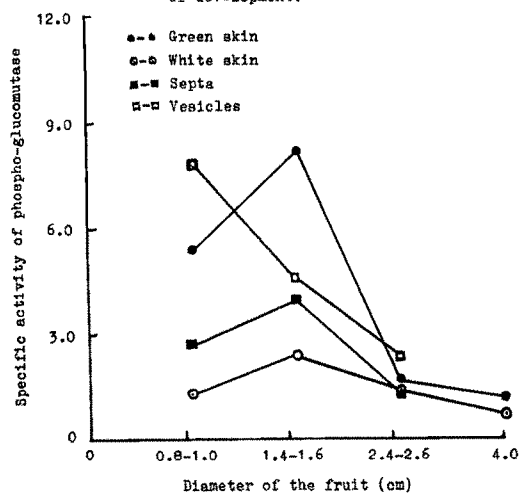
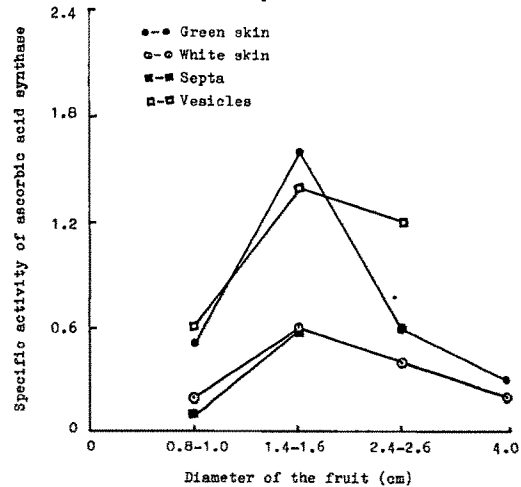


Fig. 12 : Activity of ascorbic acid synthase in different parts of the fruit tissues of *Citrus acida* at different stages of development.



These data suggest that the green skin, white skin, septa and vesicles are capable of glycolysing sugar to varying degrees. As the operation of the TCA cycle has also been shown in the case of vesicles (Ramakrishnan and Varma, 1959), it would seem reasonable to presume that the fruit tissue particularly the vesicle has the machinery to utilize sugar via the glycolytic and respiratory cycles. If the operation of the TCA cycle can be shown in the case of green and white skins and septa it would show the independence of these parts regarding the utilization of glucose for respiration.

It is also seen that the tissues showing glycolysis show a high activity of the relevant enzymes at early stages of development of the fruit when the activities of enzymes involved in citrate synthesis are low and a low activity at later stages of development when citric acid accumulates and the citrate synthesizing enzymes increase in activity.

Regarding ascorbic acid metabolism data on ascorbic acid synthesizing enzyme should be discussed with the reservations discussed earlier. The data presented in Tables 22-24 and Figs. 11-13 show the change in activity in phosphoglucomutase, ascorbic acid synthesizing enzyme and ascorbic acid oxidase in different parts of fruit tissues at different stages of the development. The juice does not show activity with regard to any of these enzymes. Regarding phosphogluco-

Table 22. Activity of phosphoglucumutase in different parts of the fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of phosphoglucumutase				
	Diameter of the fruit (cm)				
	0.8-1.0	1.4-1.6	2.4-2.6	4.0	
Green skin	5.4 (5.2,5.4,5.6)	8.2 (7.9,8.2,8.5)	1.7 (1.4,1.6,2.1)	1.2 (0.9,1.2,1.5)	
White skin	1.3 (1.2,1.3,1.4)	2.4 (2.2,2.3,2.7)	1.4 (1.2,1.4,1.6)	0.7 (0.6,0.7,0.8)	
Septa	2.7 (2.2,2.8,3.1)	4.0 (3.7,4.0,4.3)	1.3 (1.0,1.1,1.8)	N.D.	
Vesicles	7.8 (7.1,7.9,8.4)	4.6 (3.8,4.5,5.5)	2.3 (2.1,2.3,2.5)	N.D.	
Juice	N.D.	N.D.	N.D.	N.D.	

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Table 23. Activity of ascorbic acid synthase in different parts of the fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of ascorbic acid synthase				
	Diameter of the fruit (cm)				
	0.8-1.0	1.4-1.6	2.4-2.6	4.0	
Green skin	0.5 (0.3,0.5,0.7)	1.6 (1.5,1.6,1.7)	0.6 (0.5,0.6,0.7)	0.3 (0.2,0.3,0.4)	
White skin	0.2 (0.1,0.1,0.4)	0.6 (0.5,0.5,0.8)	0.4 (0.3,0.4,0.5)	0.2 (0.1,0.2,0.3)	
Septa	0.1 (0.06,0.16,0.08)	0.6 (0.4,0.6,0.8)	N.D.	N.D.	
Vesicles	0.6 (0.5,0.6,0.7)	1.4 (1.1,1.4,1.7)	1.2 (0.6,1.4,1.6)	N.D.	
Juice	N.D.	N.D.	N.D.	N.D.	

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Table 24. Activity of ascorbic acid oxidase in different parts of the fruit tissues of Citrus acida at different stages of development.

Part of the fruit	Specific activity of ascorbic acid oxidase			
	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	19.0 (16.6, 17.4, 23.0)	19.0 (16.6, 18.4, 21.8)	9.2 (8.2, 8.4, 11.0)	9.0 (8.2, 8.6, 10.2)
White skin	12.4 (11.7, 12.3, 13.2)	14.4 (13.3, 13.9, 16.0)	33.0 (30.0, 31.1, 37.9)	19.0 (16.4, 17.3, 23.3)
Septa	0.5 (0.28, 0.6, 0.62)	0.5 (0.35, 0.37, 0.78)	0.6 (0.4, 0.58, 0.82)	N.D.
Vesicles	9.0 (7.9, 8.5, 10.6)	12.0 (8.4, 12.8, 14.8)	14.0 (11.3, 15.2, 15.5)	8.2 (6.9, 8.4, 9.3)
Juice	N.D.	N.D.	N.D.	N.D.

N.D. = Not detected.

Mean of three trials. Individual values are given in the parentheses.

Fig. 13 : Activity of ascorbic acid oxidase in different parts of the fruit tissues of *Citrus acida* at different stages of development.

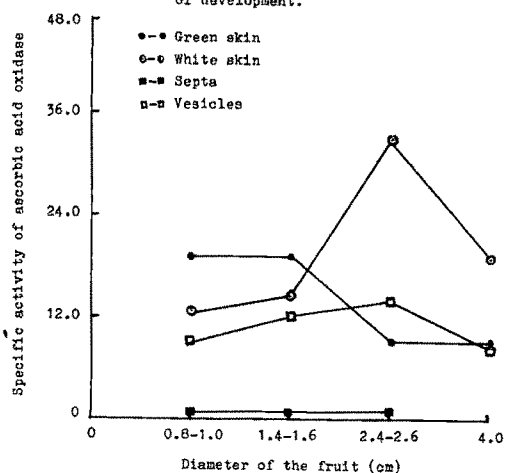


Fig. 14 : Percent dry weight of different parts of the fruit tissues of *Citrus acida* at different stages of development.

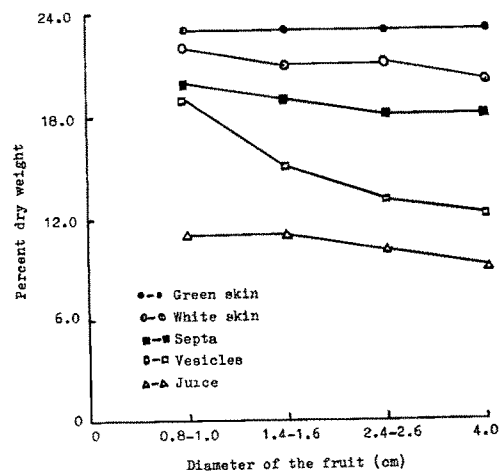


Fig. 15 : Total sugar content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

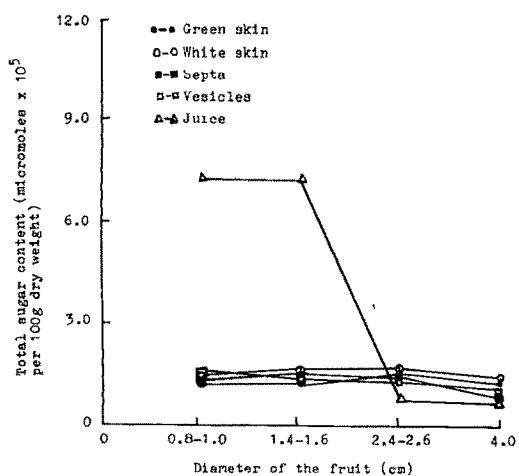
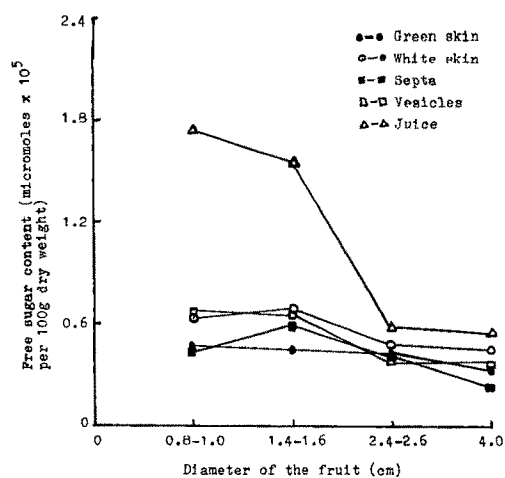


Fig. 16 : Free sugar content of different parts of the fruit tissues of *Citrus acida* at different stages of development.



mutase (Table 22 and Fig. 11) the vesicles and green skin seem to possess more activity than the septa and the white skin and the same decreases with the development of the fruit. The same picture has been observed also in the case of ascorbic acid synthesizing enzyme system (Table 23 and Fig. 12).

Regarding ascorbic acid oxidase (Table 24 and Fig. 13) the green skin and white skin show more activity than the vesicles whereas the septa shows negligible activity. This suggests the possibility that ascorbic acid plays a role in respiration in these tissues.

In conclusion the green skin and vesicles appear to be fairly active sites of carbohydrate metabolism. The higher activity of the glycolytic enzymes as well as enzymes involved in ascorbic acid synthesis and utilization in these tissues, particularly in the younger fruit, in which citric acid does not accumulate may indicate that glucose is glycolysed and utilized for ascorbic acid formation in the early stages of development of the fruit and the decrease in ascorbic acid enzymes during the later stages of development when citric acid accumulates suggests that during this stage glucose is preferentially used for citric acid synthesis.

As mentioned earlier additional studies were carried out on the concentration of the intermediates of glycolytic

cycle and ascorbic acid metabolism in different parts of the fruit at different stages of development.

Since it would be better to express all the data on the basis of per cent dry weight, first of all the dry weights of different parts of citrus fruit at different stages of development were estimated. Data given in Table 25 and Fig. 14 show that the percentage of dry weight remains almost constant in the case of the green and white skins and septa at different stages of development. Tables 26-28 (Figs. 15-17) give the total sugar, free sugar and glucose content of different parts of the fruit at different stages of development. In the case of green skin and white skin total sugar content does not seem to vary appreciably during the development of the fruit. In the case of vesicles, septa and juice there is a drop in total sugar as the fruit gets larger.

Regarding free sugar (Table 27) there is a general tendency for the same to decrease as the fruit attains a diameter of 2.4 cm or more, the decrease being more evident in the vesicles, septa and juice. The same tendency is seen with regard to glucose content except in the case of the white skin.

The differences between the green and white skin on the one hand and the septa and the vesicles on the other

Table 25. Percent dry weight of different parts of the fruit tissues of Citrus acida at different stages of development.*

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	²³ (20.6, 25.4)	²³ (21.1, 24.9)	²³ (21.3, 24.7)	²³ (22.8, 23.2)
White skin	²² (21.2, 22.8)	²¹ (20.1, 21.9)	²¹ (19.1, 22.9)	²⁰ (19.3, 20.7)
Septa	²⁰ (19.1, 20.9)	¹⁹ (18.6, 19.4)	¹⁸ (17.8, 18.2)	¹⁸ (17.6, 18.4)
Vesicles	¹⁹ (18.2, 19.8)	¹⁵ (14.3, 15.7)	¹³ (11.8, 14.2)	¹² (11.0, 13.0)
Juice	¹¹ (10.3, 11.7)	¹¹ (9.2, 12.8)	¹⁰ (9.4, 10.6)	⁹ (8.6, 9.4)

*Mean of two trials. Individual values are given in ^{the} parentheses.

Table 26. Total sugar content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles x 10⁵ per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)		
	0.8-1.0	1.4-1.6	2.4-2.6
Green skin	1.35 (1.28,1.42)	1.35 (1.32,1.37)	1.53 (1.34,1.71)
White skin	1.52 (1.48,1.55)	1.62 (1.58,1.65)	1.60 (1.56,1.64)
Septa	1.47 (1.43,1.50)	1.62 (1.54,1.69)	1.42 (1.39,1.44)
Vesicles	1.53 (1.47,1.58)	1.48 (1.45,1.51)	1.22 (1.14,1.30)
Juice	7.35 (6.35,8.34)	7.37 (7.25,7.49)	0.786 (0.773,0.799)

* Mean of two trials. Individual values are given in ^{the} parentheses.

Table 27. Free sugar content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles $\times 10^5$ per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	0.48 (0.46,0.50)	0.47 (0.42,0.51)	0.44 (0.42,0.45)	0.34 (0.29,0.39)
White skin	0.665 (0.66,0.67)	0.705 (0.70,0.71)	0.505 (0.50,0.51)	0.470 (0.45,0.49)
Septa	0.47 (0.43,0.50)	0.61 (0.53,0.69)	0.42 (0.39,0.44)	0.24 (0.21,0.27)
Vesicles	0.67 (0.61,0.72)	0.68 (0.58,0.77)	0.41 (0.38,0.44)	0.36 (0.33,0.39)
Juice	1.74 (1.54,1.94)	1.56 (1.49,1.63)	0.60 (0.60,0.60)	0.57 (0.40,0.73)

* Mean of two trials. Individual values are given in the parentheses.

Table 28. Glucose content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles x 10⁵ per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	0.030 (0.025,0.035)	0.018 (0.016,0.019)	0.0175 (0.017,0.018)	0.015 (0.013,0.017)
White skin	0.036 (0.031,0.041)	0.032 (0.031,0.033)	0.032 (0.030,0.033)	0.030 (0.029,0.031)
Septa	0.064 (0.055,0.073)	0.047 (0.038,0.056)	0.031 (0.027,0.034)	0.026 (0.025,0.027)
Vesicles	0.057 (0.048,0.066)	0.048 (0.040,0.056)	0.039 (0.032,0.045)	0.020 (0.019,0.021)
Juice	0.30 (0.22,0.38)	0.12 (0.10,0.14)	0.105 (0.10,0.11)	0.10 (0.09,0.11)

* Mean of two trials. Individual values are given in the parentheses.

Fig. 17 : Glucose content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

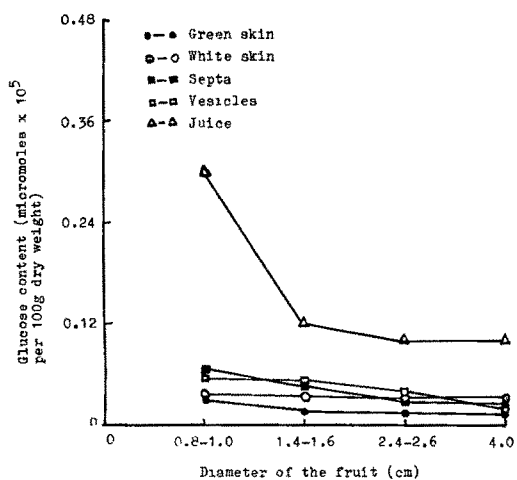


Fig. 18 : Glucose-6-phosphate content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

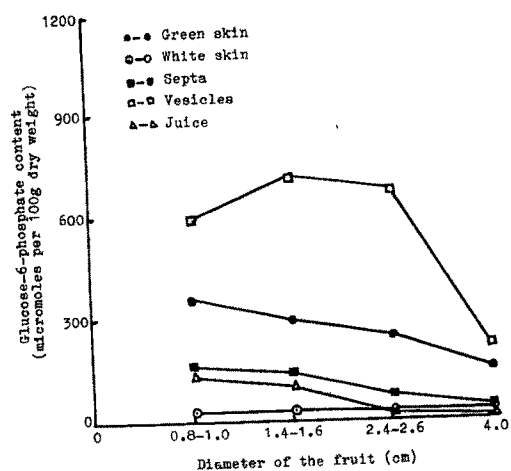


Fig. 19 : Fructose-6-phosphate content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

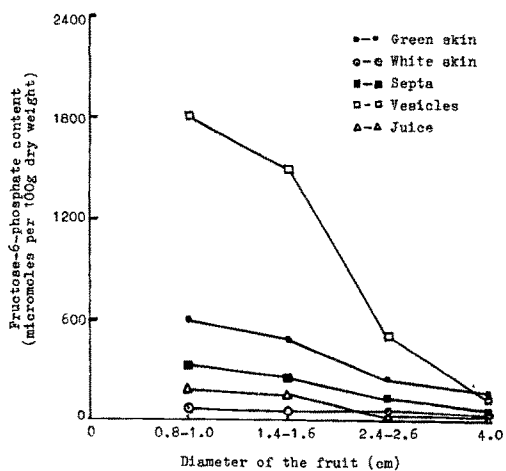
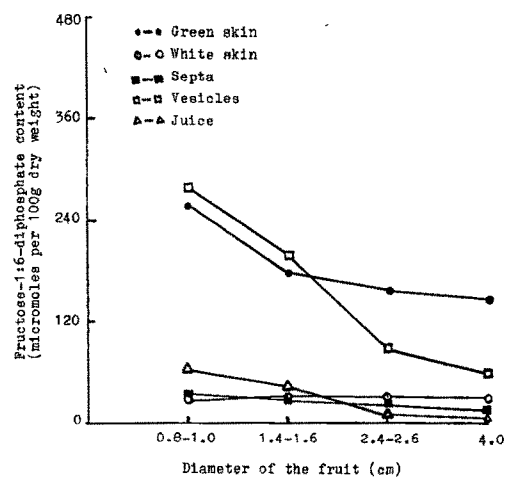


Fig. 20 : Fructose-1:6-diphosphate content of the fruit tissues of *Citrus acida* at different stages of development.



with regard to the pattern of change in total and free sugar content can perhaps be explained by those in photosynthesis as well as the rate at which carbohydrate is utilized. In other words, the photosynthetic capacity of the green skin may keep up with the sugar metabolized or translocated to other regions. In the white skin translocation from the green skin as well as the low activities of glycolytic enzymes would account for more or less steady sugar content.

The decrease in sugar content in the septa and vesicles with the development of the fruit may be due to the rate of its utilization for glycolysis and ascorbic acid and citric acid synthesis being greater than its supply from the green skin.

Since the juice is devoid of glycolytic enzymes the presence of sugars in the same must be taken to represent the amount secreted from the vesicles.

Table 29 and Fig. 18 show the glucose-6-phosphate content of the different parts of the fruit at different stages of development. The concentration decreases in the order vesicles, green skin, septa and white skin. In the former three it decreases with the development of the fruit whereas in the white skin there is not much change.

The higher concentration of glucose-6-phosphate in the green skin and vesicles can be explained partly in terms of higher concentrations of hexokinase. The low concentration

Table 29. Glucose-6-phosphate content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	360 (312,408)	305 (280,330)	250 (245,255)	150 (125,175)
White skin	25 (22,28)	25 (20,30)	22 (18,26)	18 (14,22)
Septa	161 (149,173)	140 (115,165)	70 (60,80)	30 (24,36)
Vesicles	600 (550,650)	720 (620,820)	680 (650,710)	214 (185,243)
Juice	130 (121,139)	100 (92,108)	18 (14,22)	6 (5.5,6.5)

* Mean of two trials. Individual values are given in parentheses.

in the septa can perhaps be explained in terms of a low concentration of hexokinase activity. That in the white skin, however, cannot be similarly explained and could possibly be due to the more rapid translocation of this intermediate or its further metabolism.

The data presented in Table 30 and Fig. 19 show that in the fruit fructose-6-phosphate content decreases in the order vesicles, green skin, septa and white skin. In all the cases except the white skin it decreases with the development of the fruit which can be explained in terms of the decrease in phosphohexose isomerase. In the white skin there is not much change either in fructose-6-phosphate or phosphohexose isomerase content.

Fructose-1,6-diphosphate content of different parts of the fruit at different stages of development is given in Table 31 and Fig. 20. It decreases in the order vesicles, green skin, juice, septa and white skin. While it decreases with development of the fruit in the case of green skin, vesicles and septa, it remains constant in case of white skin. This is consistent with the pattern of phosphofructokinase activity in these tissues.

Table 32 and Fig. 21 give the lactic acid content of different parts of the fruit at ^{different} stages of development. Its

Table 30. Fructose-6-phosphate content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	600 (550,650)	500 (450,550)	260 (248,272)	160 (145,175)
White skin	65 (52,78)	55 (50,60)	55 (45,65)	58 (52,64)
Septa	336 (320,352)	260 (214,306)	140 (126,154)	60 (56,64)
Vesicles	1800 (1540,2060)	1500 (1250,1750)	508 (455,560)	140 (118,162)
Juice	195 (180,210)	160 (150,170)	35 (32,38)	10 (9,11)

* Mean of two trials. Individual values are given in the parentheses.

Table 31. Fructose-1,6-diphosphate content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	²⁶⁰ (220,300)	¹⁸² (140,224)	¹⁶² (150,174)	¹⁵⁰ (137,163)
White skin	³² (25,39)	³¹ (27,35)	³¹ (30,32)	³¹ (27,35)
Septa	³³ (27,39)	³⁰ (20,40)	²⁵ (21,29)	¹⁶ (14,18)
Vesicles	²⁸⁰ (260,300)	²⁰⁰ (175,225)	⁹⁰ (85,95)	⁶⁰ (47,73)
Juice	⁶⁵ (62,68)	⁴⁴ (42,46)	¹⁰ (9,11)	⁶ (5.5,6.5)

* Mean of two trials. Individual values are given in parentheses.

Table 32. Lactic acid content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	3650 (2750,4550)	3220 (2940,3500)	1980 (1800,2160)	1500 (1375,1625)
White skin	1500 (1250,1750)	1205 (1150,1260)	1203 (1150,1256)	757 (727,787)
Septa	922 (890,954)	490 (350,630)	488 (455,520)	260 (220,300)
Vesicles	2240 (2000,2480)	1500 (1450,1550)	884 (858,910)	550 (520,580)
Juice	3100	2598	1440	912

* Mean of two trials. Individual values are given in the parentheses.

Fig. 21 : Lactic acid content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

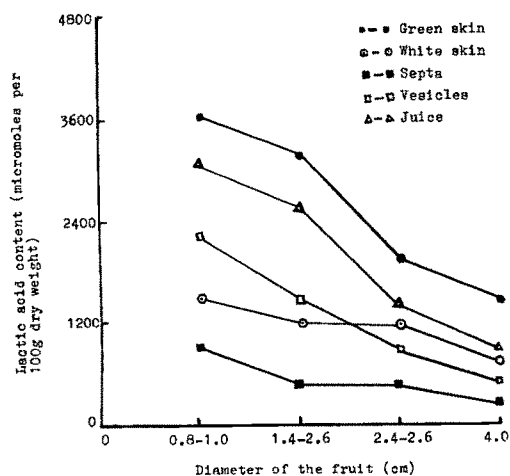


Fig. 22 : Citric acid content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

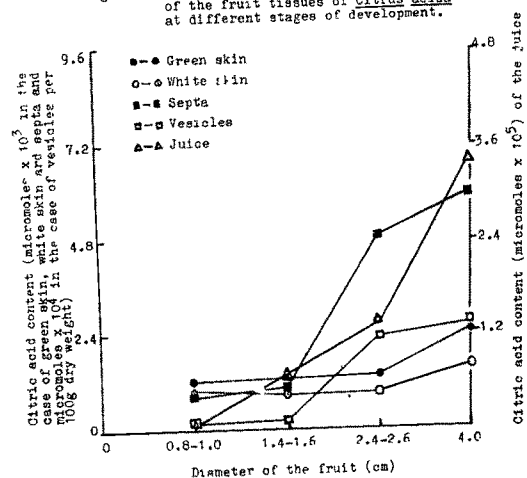


Fig. 23 : Glucose-1-phosphate content of different parts of the fruit tissues of *Citrus acida* at different stages of development.

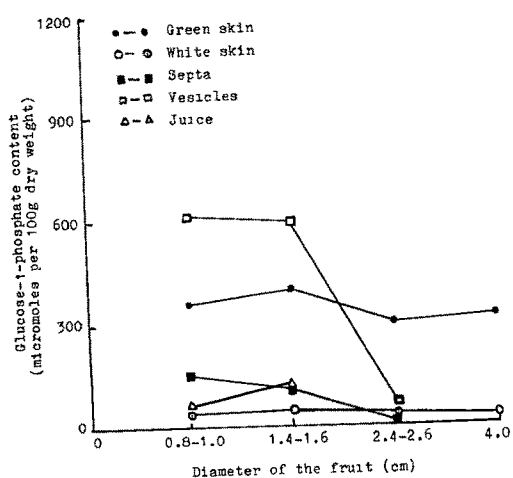
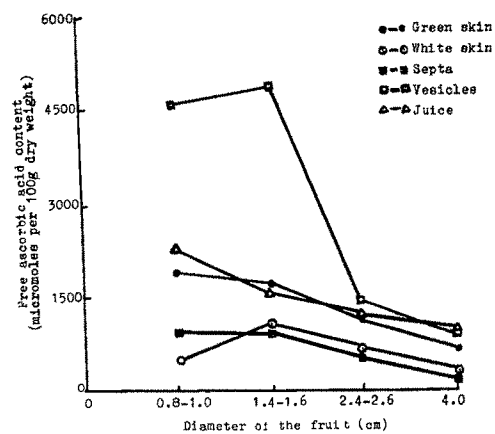


Fig. 24 : Free ascorbic acid content of different parts of the fruit tissues of *Citrus acida* at different stages of development.



concentration decreases in the order green skin, vesicles, white skin and septa. In all cases it decreases with the development of the fruit. While this cannot be explained in terms of the lactate dehydrogenase content of the tissues it is found that the activity of pyruvate oxidase in the vesicles which may indirectly control the availability of the substrate for lactate dehydrogenase increases with the development of the fruit (Parekh et al, unpublished data).

In all cases, the concentrations of the intermediates are expectedly low which would be consistent with them being metabolized continuously.

In conclusion, from the data obtained on glycolytic enzymes and intermediates it can be seen that the vesicles and the green skin possess an active glycolytic machinery and the septa, a moderate one. Comparatively speaking, the white skin appears to be a metabolically inert region. The absence of enzymes in the juice and the presence of metabolites suggest that the latter represents the spill-over from the tissues from which it is secreted.

Table 33 and Fig. 22 gives the citric acid content of the different parts of citrus fruit at different stages of development. The citric acid content of vesicles increases 15 fold in the mature fruit, 7 fold in the septa and 2 fold in the green and white skins. It has been previously mentioned

Table 33. Citric acid content of different parts of the fruit tissues of Citrus acid at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	1203 (1189,1216)	1345 (1276,1414)	1475 (1451,1499)	2499 (2417,2581)
White skin	888 (847,929)	878 (868,888)	924 (766,1081)	1631 (1436,1826)
Septa	866 (849,883)	1032 (915,1148)	4949 (4207,5690)	6070 (5121,7019)
Vesicles	1603 (1241,1964)	2638 (2380,2896)	23530 (23460,23600)	27020 (24650,29390)
Juice	6010 (5843,6176)	66660 (44040,89280)	137850 (128900,146800)	343050 (296200,389900)

*Mean of two trials. Individual values are given in ^{the} parentheses.

that during the development of the fruit sugar content decreases to a considerable extent in the vesicles and septa. The fact that the vesicles also possess high glycolytic activity and enzymes concerned with utilization of pyruvate for citrate synthesis (Parekh et al, unpublished) suggests that the vesicles contain an efficient machinery for the formation as well as accumulation of citric acid. The low concentration of citric acid in the green skin inspite of its high glycolytic activity may be due to the possibility that there is not a block for the further metabolism of citric acid. In the whole mature fruit a high activity of citrate synthase is combined with a low activity of aconitate hydratase. Possibly, this may be true of the vesicles but not the green skin. Further studies are needed to substantiate this. It is possible that the primary function of the green skin is photosynthesis and it has got glycolytic and TCA cycle enzymes to metabolise sugar for respiratory purposes and the synthesis of ascorbic acid.

Tables 34-36 and Figs. 23-25 give the glucose-1-phosphate, free ascorbic acid and dehydroascorbic acid contents of the different parts of the fruit at different stages of development. Glucose-1-phosphate decreases in the order vesicles, green skin, septa and white skin. It decreases with development in the vesicles and septa. The low amounts in the septa and

Table 34. Glucose-1-phosphate content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	360 (312,408)	400 (378,422)	300 (280,320)	320 (275,365)
White skin	38 (35,41)	48 (42,54)	35 (27,43)	28 (24,32)
Septa	150 (140,160)	112 (98,126)	21 (19,23)	N.D.
Vesicles	615 (580,649)	600 (550,650)	73 (65,81)	N.D.
Juice	65 (60,70)	120 (115,125)	N.D.	N.D.

N.D. = Not detected.

* Mean of two trials. Individual values are given in the parentheses.

Table 35. Free ascorbic acid content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	1903 (1846, 1960)	1752 (1704, 1800)	1165 (1080, 1250)	710 (653, 767)
White skin	500	1136	710	341
Septa	943 (897, 988)	954 (897, 1011)	597 (540, 653)	227 (181, 272)
Vesicles	4557 (4261, 4852)	4858 (4716, 5000)	1482 (1363, 1600)	983 (926, 1040)
Juice	2278 (2000, 2556)	1709 (1600, 1818)	1250 (1136, 1363)	1023 (909, 1136)

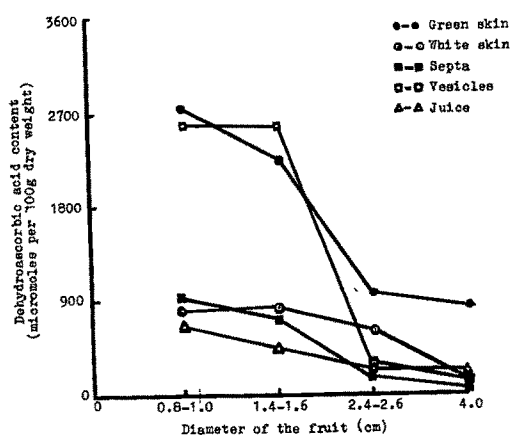
* Mean of two trials. Individual values are given in the parentheses.

Table 36. Dehydroascorbic acid content of different parts of the fruit tissues of Citrus acida at different stages of development.*
(Values expressed as micromoles per 100g dry weight)

Part of the fruit	Diameter of the fruit (cm)			
	0.8-1.0	1.4-1.6	2.4-2.6	4.0
Green skin	2722 (2671, 2773)	2228 (1989, 2466)	1023 (852, 1193)	853 (682, 1023)
White skin	818	852	625	125
Septa	943 (920, 966)	716 (659, 773)	239 (205, 273)	63 (51, 74)
Vesicles	2597 (2415, 2778)	2558 (2415, 2700)	347 (341, 352)	126 (120, 131)
Juice	684 (600, 767)	483 (443, 522)	312 (272, 352)	255 (238, 272)

* Mean of two trials. Individual values are given in the parentheses.

Fig. 25 : Dehydroascorbic acid content of different parts of the fruit tissues of *Citrus* acida at different stages of development.



vesicles of the mature fruit may be due to decrease in phosphoglucomutase activity.

The concentration of free ascorbic acid decreases in the order vesicles, green skin, white skin and septa. It also decreases during later stages of development of the fruit. The activity of ascorbic acid synthesizing enzyme also follows the same pattern. But as the system used does not appear to be very efficient it would be better to reserve the comments on the relation between the enzyme concentration and ascorbic acid content.

Dehydroascorbic acid content decreases in the order green skin, vesicles, septa and white skin. It decreases in the mature fruit in all cases.

Discussion

Ramakrishnan and Varma (1959) have shown that in the edible portion of the fruits of Citrus acida glucose content decreases and citric acid increases with the development of the fruit. They have detected the TCA cycle enzymes in the young fruit tissues in which no citric acid accumulates and shown that the enzymes necessary for citric acid production, namely pyruvate oxidase and citrate synthase enzyme increase and aconitate hydratase is absent in the mature tissues in

which citric acid accumulates. Recently Sakariah et al (unpublished) have shown that protein is utilized for respiration in the mature tissues by getting broken-down into glutamic acid and being utilized via 2-oxo-glutarate thus bypassing the aconitate step in the respiratory cycle. The operation of the TCA cycle would presuppose that of the glycolytic pathway in the fruit tissues and this supposition is confirmed by the present studies. The results of the present investigation thus strengthen the hypothesis that the fruit tissue is an independent metabolic entity with a complete machinery for the metabolism of glucose.

The fruit tissue also seems to possess enzymes which can convert glucose to ascorbic acid.

Regarding the capabilities of different parts of the fruit to metabolise sugar the data collected show that vesicles and green skin are very active sites for the synthesis of ascorbic acid. The green skin seems to be an active site for the photosynthesis of sugar. The vesicles seem to be the main site of citric acid formation followed by the septa. The white skin seems to be rather a relatively inert site of carbohydrate metabolism and its major function could be translocation of glucose from the green skin to the interior of the fruit. Juice seems to represent merely the spilled-over products from the tissues.

It is interesting to note that glycolysis and ascorbic acid synthesis are high in young fruit tissues which do not accumulate citric acid and low in mature tissues in which citric acid accumulates.

Thus these studies show that different parts of the fruit tissue differ in the metabolism of carbohydrate and point to the vesicles as the main location for citric acid synthesis, green skin for photosynthesis and vesicles and green skin for ascorbic acid synthesis. Studies are under progress in this laboratory to cultivate vesicles in vitro in a medium free from host influence. Isotope studies on such cultivated vesicles are needed to confirm these findings.