BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FRUITS

Part II.—Differential Mechanism of Ripening of Ordinary Variety and *Kanchamitha* (Unripe Green Sweet) Variety of Mangoes (*Mangifera indica*)

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The differential mechanism of ripening of local ordinary varieties of mangoes has been investigated by measurement of the changes in titratable acidity, reducing and non-reducing sugars and the dehydrogense activities at different stages of their growth upto ripening and comparison against the values of the same for *Kanchamitha* variety which is sweet even in the green stage. The results show gradual increase of acidity in every part of the ordinary mango with the progress of growth upto maturity and then a decline of the above value when the mango ripens. This fall in acidity is associated with the formation of both reducing and non-reducing sugars and the elaboration of dehydrogenase activities. In the case of *Kanchamitha* variety, even in its unripe condition the acidity is low in association with more contents of sugars and more activity of the dehydrogenases. The significance of these results in the above varieties of mangoes has been discussed in the light of the two different mechanisms operating in the process of ripening of mangoes.

Introduction

In East Pakistan different varieties of mangoes are grown every year in sufficient quantities. But in the humid tropic atmospheric conditions of the region a large fraction of the total product get spoiled due to overripening and ultimate fermentative breakdown of sugars. In order to develop preservation techniques, it is necessary to have complete information about the actual mechanism of the process of ripening for each individual variety of mango. This necessitates the study of the formation of acids, gradual breakdown of acids with simultaneous formation of sugar and lastly the fermentative breakdown of sugar to alcohol, and also of the different enzyme systems which are involved in the whole chain of the above reactions. Though the overall reaction in each variety may be of the same nature, the period of onset of some enzyme systems in one variety pertaining to ripening process may be different from the other or the pathway of activities of some enzyme systems may differ from variety to variety. This possibility has been shown by Qudrat-i-Khuda, De and Khan[1] in their work on the ripening of two opposite varieties of Banana,—one "Amrit Sagar" (Gross Mitchell) and the other vegetable variety where it was shown that the dehydrogenase activity develops in the "Amrit Sagar" variety with gradual formation of sugars during ripening whereas this is completely absent in the vegetable variety which, even on storage for a long priod, does not show any formation of sugar. It is quite possible, therefore, that similar differences might also exist in different varieties of mangoes which show different degrees of sweetness and flavour after ripening. With this possibility in view, the present work has been undertaken to investigate the mechanism of ripening of one local variety of mango by study of the changes of its acidity, sugar content and the dehydrogenase activities and by their comparison against the similar values of another variety which is sweet even in unripe green stage and locally called the *Kanchamitha* Variety. (Kancha means green unripe and Mitha means Sweet)*

Work carried out on some important varieties of mangoes grown in India are reported in the literature[2-7] and most of this work refers to the difference in the acidity and sugar contents in green and ripe mangoes. But no attempts have been made to correlate these differences with the activities of some dehydrogenases, kinase etc. which may actually help to judge the differential characteristics of the various varieties with respect to the formation of acids and sugars in unripe and ripe conditions. In the present investigation attempts have been made in this direction by simultaneous study of the activities of dehydrogenases in green and ripe conditions.

Experimental

The tree of the mango of the local *Desi* variety was selected from the laboratory garden and the fruits were collected at regular intervals from the smallest edible stage upto maturity and ripeness. The sizes of the fruits at each stage of collection were measured and these are expressed as length and breadth in cm. and the averages of large number of fruits collected at each stage are shown in the Table. These were then segmented with glass edge into four fractions—(a) skin, (b) flesh or marrow (c) seed and (d) the seed cover.

*This variety is grown in East Pakistan and West Bengal and mentioned by Ganguly *et al.* on Marketing of Mangoes in India—Agri. Marketing Ser., **77** 38-454 (1958) and also referred to in Wealth of India, C.S.I.R., New Delhi, Raw Materials Vol. VI (L-M), p. 270.

The *Kanchamitha* mango being a rare variety, was procured from one of the gardens in a rural area six miles away from the laboratories. The samples after collection were quickly brought to the laboratories avoiding loss of moisture and spoilage. The fruits from the tree could be procured at only three stages of their growth. These were similarly segmented into four fractions like the ordinary mango. The following determinations were then made for each of the above fractions.

the extract with HCl and deducting the value of the reducing sugar from the hydrolysed value.

The auto-dehydrogenase activity was determined according to the method described by Qudrat-i-Khuda, De and Khan by adopting the Thunberg Methylene Blue technique[9] in which the total dehydrogenase activity of one gram ground tissue was evaluated by noting the time of discharge of Methylene Blue after incubation of the tubes at 37°C. In this modified

Table. 1—The Titratable Acidity, Sugars, and Auto-Dehydrogenase Activities in Different Segments of Common Variety and Kanchamitha Mango at Various Stages of their Growth.

Nature of mango and stage of growth	Size Length in cm.	Size Breadth in cm.	Titratable acidity as percent of malic acid: Skin	Flesh	Seed	Seed cover	The time of discharge of methylene blue in minutes for 1 g. tissue: Skin	Flesh	Seed	Seed cover	Sugar percent: Non-reducing	Reducing
Common variety green unripe	4.1	3.5	0.34	2.17	—	—	—	—	—	—	—	—
,, ,,	4.5	4.1	0.42	2.41	—	—	—	—	—	—	—	—
,, ,,	6.2	5.2	0.58	2.52	—	—	—	—	—	—	—	—
,, ,,	7.6	6.0	0.64	2.61	—	—	—	—	—	—	—	—
,, ,,	8.2	6.3	0.42	3.05	—	—	66	—	—	—	—	—
,, Mature	9.2	8.3	0.29	0.87	0.44	0.38	54	(**)	10	(**)	3.1	1.5
,, Ripe on tree	10.2	8.5	0.17	0.78	0.14	0.04	110	(**)	9	114	3.8	1.7
,, Ripened under storage	10.4	9.2	0.14	0.65	0.12	0.06	123	111	5	41	9.45	1.94
,, *Kanchamitha* variety	6.5	5.2	0.15	0.74	0.34	0.06	40	(**)	70	170	0.48	2.72
,, ,,	7.6	6.2	0.13	0.70	0.26	0.11	25	(**)	55	131	2.4	2.24
,, ,,	8.2	6.5	0.11	0.70	0.18	0.08	20	(**)	58	124	2.6	2.38

(**) The time of discharge of M.B. in such case was more than 4 hours and hence negligible activity of dehydrogenase as expressed by I/T

The acids produced in the mango consist of malic, citric, succinic, oxaloacetic, tartaric along with others and all these are measured as titratable acidity by grinding 5 g. of the tissue with a little water and glass powder, boiling, centrifuging the extract and repeating the process thrice and finally making up the volume to 25 ml. and by Titration of the aliquots against N/10 NaOH. The values of the titratable acidity are expressed as percent of malic acid.

The reducing sugar of the above extract was determined according to Lane and Eynon method [8] and the non-reducing sugar was determined by the same method after hydrolysis and

method the dehydrogenases of the tissues were allowed to act on the substrates which are supposed to be present in the tissues as partner of the dehydrogenases and the reciprocal of the time of discharge of M.B. denote the activity of the total dehydrogenases instead of representing a particular one.

In the early stage of growth of ordinary mango the time of discharge of M.B. was very low denoting lower activity. In the Table the astericks shown in the columns of dehydrogenase activity should be considered as the longer time required for discharge of Methylene Blue and hence negligible activity of dehydrogenase.

Results

Common Variety Mango.—The results presented in the Table show that acidity expressed as percent of malic acid is distributed in all the parts of mango in its green unripe as well as in ripe conditions—the maximum quantity is however, present in the flesh. The skin, seed and the seed cover also contain the titratable acids though in smaller quantities.

The titratable acidity in the fleshy portion of the common variety of mango is low at the initial stage with an average value of 2.17 percent at the size grade of 4.1×3.5 cm. The value gradually increases with the progress of growth until it reaches to a peak level of 3.05 at the maturity size grade of 8.2×6.3 cm. Above this size there is drop of the acidity which reaches to a minimum value of 0.78 percent in ripe condition. When the mango was ripened by storage after maturity the acidity decreased to a lower level of 0.65 percent. In the skin also similar phenomenon is noted but in this case peak level is reached at an earlier growth stage at the size grade of 7.6×6.2 cm. In this tissue the acidity increases from a very low level of 0.34 percent to a peak level of 0.64 percent and then drops to a minimum value of 0.14 percent in the ripe condition under storage. In the seed the acidity drops from 0.44 to 0.12 percent while passing from maturity to ripeness. The seed cover containing lesser quantity than the seed also showed similar trend in the drop of the acidity when the mangoes ripen after maturity.

The fall in the titratable acidity is accompanied by simultaneous production of both reducing and non-reducing sugars after maturity and reaches to 3.8 and 1.7 percent, respectively for the non-reducing sugar and reducing sugar when the mango ripens on the tree but higher value of non-reducing sugar of 9.48 percent is noted when the mango is ripened by storage in ordinary condition after it had reached the maturity. All the above values of acidity and sugars are almost of the same order as observed in case of other common varieties reported elsewhere.[2–7]

In the present investigations it has been further observed that the above fall in the acidity with simultaneous formation of sugar is associated with gradual elaboration of the auto-dehydrogenase activity.

Measurable activity of the enzymes was first noted in the skin (66 minutes time of discharge of methylane blue) at the size grade of 8.2×6.3 cm. just before maturity. The activity in this tissue increases at maturity but falls when the mango ripens on tree or by storage. Seed and seed cover elaborated maximum activity as evident from less time of discharge of M.B. and in contrast to that in the skin the activity in these tissues decreases with progress of ripening. In the flesh even in the mature and ripe condition the activity was negligible (**) but it showed its appearance only when the mango was ripened by storage. It is strikingly noted that this appearance of activity in flesh is associated with the drop in the activity in the skin and with formation of larger quantities of non-reducing sugars in the flesh.

Kanchamitha Mango.—It is strikingly noted in this variety of mango that even in its green unripe stage for equal size of 6.2×5.2 cm. and 7.6×6.2 cm. as those of ordinary mango the acidity is much less and this approaches to the same limit as that in ordinary mango in ripe condition. This low acidity is associated with high quantity of reducing and non-reducing sugars and considerable activity of the autodehydrogenases in their skin, seed and cover. Unlike the stored ripe mango of common variety the dehydrogenase activity in the flesh was negligible and this is associated with lower non-reducing sugar content in the tissue.

Discussion

The above findings of negligible dehydrogenase activity in the unripe mango of common variety and elaboration of their activities with fall in acidity and simultaneous production of sugars in their ripe condition coupled with the results of *Kanchamitha* mango where the auto-dehydrogenase activities are elaborated even in the green stage in association with sugar formation with less acidity lead us to suggest that a number of dehydrogenase enzymes are involved in the synthesis of sugars—both reducing and non-reducing, but not much in the formation of acids.

In tracing out the differential metabolic behaviour in the above two varieties of mangoes, it will be worthwhile to discuss the whole aspect on the basis of investigations carried out by a group of workers as described by Balsham and Calvin,[11] Thomas and others[12] who explored the mechanism of carbon reduction in photosynthesis with the help of tracer technique. It has been proved that some acceptor already present in the storage tissues fixes CO_2 with production of phosphoglyceric acid (PGA) which is now considered as the first product of photosynthesis. This PGA may then follow two courses: either by reduction it may be converted to Triose phosphate and proceed in a reverse direction of Embden Meyerhof

pathway giving rise to hexose and pentose sugars and sucrose with involvement of pentose sedoheptulose cycle and UDG—UDPG systems [13–15] or it may be converted to phosphopyruvic acid by enolase which may then give rise to different acids like malic, succinic, citric etc. by entering the Kreb's cycle and also by fixation of CO_2 to pyruvic acid giving rise to malic acid and oxaloacetic acid. While scrutinising the different enzyme systems involved in the above two processes it will be evident that 4 more dehydrogenases are involved in the pathway for the formation of carbohydrates, whereas less is involved in the production of acids.

In the perspective of the above information, it may be suggested that in case of common variety of mango in their unripe condition upto the stage of maturity, the PGA after its formation perhaps mainly follows the second pathway i.e. the formation of acids through pyruvic acid and Kreb's cycle with the net result of gradual increase of acidity with progress of growth. During this phase perhaps the first pathway i.e. the reduction of PGA to triose phosphate remains blocked. After maturing some of the acids like succinic, malic and oxadoacetic may now proceed in reverse direction through phosphopyruvic acid, PGA and then to triose phosphate and ultimately in the production of different sugars according to the reaction as discussed above with involvement and a number of dehydrogenases and oxidases. In the case of *Kanchamitha* variety the position seems to be different. Here perhaps the first pathway in the conversion of PGA to triose phosphate is more active with formation of sugars even in its green stage. As most of the PGA formed in this mango follows this first pathway a small fraction is perhaps left to proceed through the second pathway with the formation of small quantity of acid as is observed in the present investigation. Further investigations are being carried out to make comprehensive studies of the various enzymes and probable inhibitors involved in the mechanism of ripening of the ordinary mango vis-a-vis *Kanchamitha* variety and that the knowledge and informations gathered may be applied for delaying or hastening of ripening of mangoes as may be desired.

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