BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN BANANA

Part I.—Investigation on the Mechanism of Banana Ripening by Evaluation of their Auto-Dehydrogenase Activities

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The autodehydrogenase activity of the banana *M. Dacca, Horan,* locally called 'Amrit Sagar,' was studied on different batches attached to and detatched from the stalk during the process of ripening under storage at ordinary room temperature. Side by side the activity in plantain, i.e. cooking banana (*M. paradiciaca*), which does not ripen easily but takes longer period of storage, has also been studied under the same storage conditions. In the case of 'Amrit Sagar' variety it has been observed that bananas attached to the stalk ripened more quickly and elaborated more dehydrogenase activities than those detached from the stalk. In all the batches whether stalked or destalked, the above enzymes were concentrated more in the peel than in the pulp. Among the different regions of the peel and the pulp, the stylar end elaborated more activities than the stem end portion. The significance of the above findings were discussed in the light of the glycolytic breakdown of starch to sugar and other constituents during the different phases of ripening.

Introduction

East Pakistan produces nearly 1.6 million tons of different varieties of banana every year. Among these, the 'Amrit Sagar' variety grows in largest quantity and is popular not only in this country but also in other neighbouring countries for its sweet flavour, softness of the seed-free flesh and other qualities. The cultivation of this variety of banana was previously localised in some regions in the District of Dacca in East Pakistan and perhaps *Sapeintum var Dacca Hort* (M. Dacca, Horan) refers to the above 'Amrit sagar banana.' Watts [1] has described this as 'Dhakai Kela', i.e. and banana originating from Dacca.

A large portion of this and other varieties of banana grown in this region get spoiled during transport by country boat and by storage under ordinary conditions, resulting in a loss of nutritional value. This happens because of the rapidity of the processes through which the different phases of ripening proceed in this tropical, and humid climatic condition of the region.

In many countries of the West and of the East a great deal of work has been done on the control of ripening and delaying its onset during transport and storage and all the work has been reviewed by von Loesecke[2] and others. But no work has yet been done in this country where the problem is much more complicated because of the humid and tropical climatic condition of the region. It was, therefore, thought desirable to study the whole field of the mechanism of ripening and spoilage of different varieties of banana grown in this region so that an economic method or methods may be developed for their transport and storage with curtailment in the loss of the nutritive value due to spoilage.

Storage by chilling in low oxygen and high carbon dioxide atmosphere and by such other methods as adopted in Western countries presents a number of difficulties. Gas storage with CO_2 sometimes deteriorates the quality and produces 'gas injury' characterised by darkening of the skin.[3,4] Storage by extreme chilling causes complete retardation of ripening with formation of dark spots on the skin.[5-8] All the above processes are based on the principle of limiting the hydrolysis of starch either by controlling the respiration with the help of carbon dioxide atmosphere or by inhibition of enzyme activities by chilling.

On survey of the whole process of ripening it is revealed that this is constituted by three separate phases—preclimacteric, climacteric and senescence phases and at each phase different constituents are generated. In reviewing the literature so far collected it appears that the preclimacteric phase is associated with low R. Q. and this is followed by high R. Q. in the climacteric phase. In the senescence phase there is drop of the R.Q. although at a slower rate. The R.Q. again shows a sharp rise if the banana is allowed to overripe and undergo spoilage.[1,9-11] The above changes in the R.Q. are accompanied by gradual breakdown of starch with the production of sucrose, glucose and fructose as has been noted by many workers.[1,12-14] During the breakdown of starch, there is some loss of the total carbohydrates (10 to 15%) and this may be accounted for by simultaneous production of malic, oxalic, citric, succinic and other acids with the fall in the pH value of the pulp [15-20] and of acetaldehyde, amyl acetate etc. leading to aroma [1,21] and formation of anthocyanin, flavones, carotene and anthophyll pigments with simultaneous decrease in the chlorophyll content leading to the production of yellow to brown or reddish colour.[22]

If the bananas are allowed to overripe or undergo spoilage, part of the sugar formed is fermented to ethyl alcohol.[1] Moreover, for acquiring energy for the operation of the different enzymatic processes, part of the starch at the initial stage is directly converted to carbon dioxide and thus cannot be accounted for.

All the above observations lead to postulate that the stored starch in the mature but unripe banana undergoes similar sequences of aerobic and anaerobic degradation by a chain of enzyme systems as are involved in the breakdown of muscle and liver glycogen. Various works showing the involvement of similar pattern of enzymatic pathways in the breakdown as well as in the synthesis of glycogen in the human body and of starch in the plant kingdom, have been reviewed elsewhere.[25] Calvin's recent studies as to the involvement of some more hitherto unknown enzyme systems in the synthesis and breakdown of starch, have added new knowledge to this filed.[24,25] In the background of this information, it will be worthwhile to study the various enzyme systems involved in the different phases of banana ripening and spoilage, so that newer information may be gathered which may help to control the ripening process in a more systematic manner by use of some enzyme inhibitors or activators as may be needed at certain stage of the ripening process.

A series of investigations have, therefore, been started in these Laboratories and in the first of these, the study of the dehydrogenases which are involved in the glycolytic or (amylolytic) breakdown of starch to pyruvic acid and in the Kreb's tricarboxylic acid cycle, has been undertaken with 'Amrit sagar' banana during their storage under ordinary conditions. Similar study has also been made with the plantain or cooking banana (*M. paradiciaca*) which is consumed in this region as vegetable in curries and locally called 'kacha kela' which means green (kacha) banana (kela). This variety generally does not ripen like other varieties under ordinary storage condition but does so very slowly if stored for a prolonged period and it was expected that simultaneous study of the above enzymes in this variety might throw new light to correlate the ripening process with the dehydrogenase and other enzyme activities.

Experimental

Two stalks of the above species of banana, with a number of bunches in each, were collected at their proper maturity in the green condition. A few bunches from each of the stalks were separated from the stalk and the rest were left attached to the stalk to see how far the detachment or attachment to the stalk influences the ripening process and the enzyme activity. These were then hung in a small glass-walled cupboard with an air access at the top and at the bottom. At the initial stage, few samples from the destalked and the stalked bunches were removed from the rest. After each 24-hour storage similar samples were removed from each of the bunches. Circular discs of 1 cm. thickness were sliced from the stylar end, middle and the stem end portions of each sample and the peel and the pulp portions were then separated and the autodehydrogenase activities of these portions were then estimated according to the Thunberg technique described in the pervious report by the authors[26] in their investigation on the mechanism of fish spoilage. The technique is based on the measurement of the time of discharge of methylene blue dye under vacuum in Thunberg tube containing 1 g. tissue extract in phosphate buffer of pH 7.8. According to this technique the dehydrogenases were allowed to act upon the substrates which were supposed to be present in the tissues along with the enzymes.

The above batches ripened at different periods of storage under laboratory temperature of 80-85 °F. and their enzyme activities were studied up to the periods mentioned in Table 1.

TABLE 1

Variety	Condition of storage	Period of storage in days	Remarks
Amrit sagar	Attached to the stalk	5 (fully ripened)	Spoilage started after this period. Enzyme activities studied up-to the ripening period
,,	Detatched from the stalk	8 ,,	
Plantain	Attached to the stalk	15 (not ripened)	Slight yellow colour of the peel on the 12th day.
,,	Detatched from the stalk	15 ,,	No change in colour even after 15 days. Enzyme activity was studied up to 15 days in both batches.

Results and Discussion

The results obtained with the 'Amrit Sagar' variety are presented in Table 2 and the figures in the table indicate the averages of the replicate values of the time of discharge of methylene blue (M.B.) per gram tissue. The enzyme activity may be calculated as the reciprocal of the above time of discharge of M. B.

TABLE 2.—THE TIME OF DISCHARGE OF THE METHYLENE BLUE DYE (M.B.) BY 1 g. TISSUE OF THE DIFFERENT PORTIONS OF PEEL AND PULP OF THE BANANA UNDER THE VARIETY *Sapientum var Dacca Hort* (*M. Dacca, Horan*) LOCALLY CALLED 'AMRIT SAGAR KELA'. THE ROOM TEMPERATURE DURING STORAGE WAS 80 - 85 °F.

Time of storage in days	Bunches attached to stalk					Hands detatched from stalk				
	Colour of the peel*	Peel (Pe) or Pulp (Pu)	Time of discharge of M.B.			Colour of the peel*	Peel (Pe) or Pulp (Pu)	Time of discharge of M.B.		
			Stylar end	Middle	Stem end			Stylar end	Middle	Stem end
0	G	Pu	← 8 hrs. →			G	Pu	← 8 hrs. →		
		Pe	← 6 hrs. →				Pe	← 6 hrs. →		
1	G - Y	Pu	2h - 20m	3h - 40m	4h - 10m	G	Pu	← 6½ hrs. →		
		Pe	1h - 10m	1h -40 m	2h - 34m		Pe	← 5 hrs. →		
2	G - Y	Pu	52m	1h - 20m	1h - 40m	G - Y	Pu	3h - 10m	3h - 40m	4h - 54m
		Pe	42m	1h - 2m	1h - 24m		Pe	2h - 2m	2h - 30m	3h - 10m
3	Y	Pu	42m	52m	1h - 10m	G - Y	Pu	2h - 40m	3h - 18m	3h - 34m
		Pe	31m	33m	1h - 3m		Pe	1h - 48m	2h - 10m	2h - 30m
4	Y	Pu	52m	46m	1h - 3m	G - Y	Pu	2h - 12m	2h - 48m	3h - 2m
		Pe	20m	32m	45m		Pe	1h - 4m	1h - 32m	1 - 58m
5	B - Y	Pu	22m	31m	48m	Y	Pu	2h - 2 m	2h - 10m	2h - 32m
		Pe	10m	15m	18m		Pe	48m	53m	1h - 6m
6	Spoilage started in the batches.					Y	Pu	1h - 20m	1h - 45m	2h - 0m
							Pe	40m	48m	56m
7						Y	Pu	55m	1h - 15m	1h - 38m
							Pe	30m	38m	52m
8						B - Y	Pu	42m	58m	1h - 10m
							Pe	20m	29m	40m

* G denotes green.
Y ,, yellow.
B ,, brown.

G - Y denotes greenish yellow.
B - Y ,, brownish yellow.

From the data it would appear that all the bananas of the bunches attached to the stalk started ripening from the 3rd day when the colour of the peel changed to yellow and further to brown yellow colour on the 5th day. After this period the bananas of these bunches showed overripening and spoilage. In the bunches detached from the stalk slow ripening started from the 5th day. In these bunches overripening and spoilage started after 8th day storage.

While surveying the data of the dehydrogenase activities, it seems evident that at the initial stage prior to storage very negligible activity was elaborated by the green banana as evident from the requirement of longer period for the discharge of the M.B. by the different portions of the peel and the pulp at that stage. The bananas in the bunches attached to the stalk showed the generation of the dehydrogenase activity after 24 hours storage and within the next 48 hours the value showed a further increase at a steeper rate after which the rate of increase of the activity slowed down until on the last day of the ripening. In the bananas of the bunches detached from the stalk the above enzymes elaborated slight activity late after 48 hours and thereafter the rate of increase of the activities proceeded very slowly up to the last day of the ripening period.

While comparing the activities of the peel with those of the pulp it is noted that at all the stages of ripening the activites were concentrated more in the peel than in the pulp. This indicates that the ripening of the pulp perhaps starts from the surface just beneath the peel due to the influence of the high level of dehydrogenases or other enzymes concentrated in the peel and then gradually proceeds towards the interior of the pulp.

Comparison of the activities located in different portions of the peel and the pulp in both the batches showed a very high concentration towards the stylar end and gradual decrease towards the stem end. This parallels with the gradation of ripening which shows an advance at the stylar end.

From the survey of the data, it is also revealed that at a particular period of storage the bunches detached from the stalk showed lower degree of ripening accompanied by lower dehydrogenase activities as compared to those attached to the stalk. On the basis of these findings it will not be unreasonable to assume that perhaps the stalk is the store house for all the enzymes involved in the ripening of bananas wherefrom these are translocated to the hands and then to the peel. The possibility of the presence of some factor in the stalk which aids in the synthesis of the enzymes in the hands for ripening or of some co-factor which aids, supplements or accelerates the activities of the ripening enzymes already synthesised in the stalk independently, cannot be overruled. Further work is in progress to explore the above possibilities.

Investigation with plantain (cooking banana) revealed complete absence or a very negligible amount of the above enzyme systems at the initial stage before storage and even after storage for a prolonged period up to 15 days as was evident from the consumption of more than 12 hours for the discharge of the dye M.B. In case of the bunches attached to the stalk slight activity was noted after 12 days storage as was evident from the consumption of only 3 hrs. for discharge of M.B. dye. Since the activity of dehydrogenases in all the batches was not very significant, the data for plantain were, therefore, not shown in the table. But it is apparently true that the non-ripening of plantain for prolonged storage under similar conditions is due to absence of the dehydrogenase and other enzymes which are involved in the breakdown of starch to sugar and other constituents. A thorough investigtion has now been started to see if the above apparent absence of the deydrogenases in the plantain is due to complete absence of the mechanism for the synthesis of the above and other enzymes in this variety or due to blockage of the activities of these enzyme systems by some inhibitor or inhibitors.

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References

1. G. Watts, Dictionary of the Economic Products of India, **V**, 290 (1891).
2. H. W. von Loescke, *Banana* (1950) Interscience Publication.
3. E. R. Leonard, Trop. Agr. (Trinidad), **28**, 190 (1946).
4. C. W. Wardlaw, Imp. Coll. Trop. Agr., Trinidad, Mem. Low Temp. Research Sta. No. 15 (1940), Trop. Agr. (Trinidad), **17**, 103 (1943).
5. E. R. Leonard and H. R. Barnell, Imp. Coll. Trop. Agr., Trinidad, Mem. Low Temp. Research Sta. No. 11, (1943).
6. H. R. Barnell, Ann. Botany, (London), **7**, 1, (1943).
7. Idem., ibid, **5**, 608, (1941).
8. E.R. Leonard, Proc. 8th Intern. Congr. Refrig., London, (1951) p. 647.
9. G. L. Poland and R. M. Wilson, United Fruit Co., Research Department, Bulletin No. 46 (1933).
10. E. R. Leonard, Ann. Botany (London), **5**, 89 (1941).

11. R. Gane, New Phytologist, **35**, 383, (1936).
12. F. C. Stratton and H. W. Loesecke, United Fruit Co., Research Department, Bulletin No. 32 (1930).
13. H. R. Barnell, Ann. Botany (London), **5**, 217, 608 (1941).
14. G. L. Poland, J. T. Manion, M. W. Brenner and P. L. Harris, Ind. Eng. Chem., **30**, 340 (1938).
15. W. D. Bigelow and P. B. Dunbar, Ind. Eng. Chem., **9**, 762 (1917).
16. B. G. Hartmann and F. Hillig, J. Assoc. Offic. Agri. Chemists, **17**, 522, (1934).
17. E. F. Kohman, J. Nutrition, **18**, 233, (1939).
18. C. Fonseka, Rev. alimentar (Rio de Janeiro), **5**, No. 37 , 29 (1941).
19. P. L. Harris and G. L. Poland, Food Research, **2**, 135 (1937).
20. H. Brunner and E. Chuaard, Ber, **19**, 595 (1886).
21. C. Klebber, Am. Perfumer, **7**, 235, (1913).
22. H. W. von Loesecke, J. Am. Chem. Soc., **51**, 2439 (1929).
23. W. G. James, Advances in Enzymol., **18**, 281, (1957).
24. J. A. Bassham and M. Calvin, *Currents in Biochemical Research* (1956), p. 29.
25. M. Calvin, Proc. 3rd Intern. Congr. Biochem., (1956), p. 211.
26. M. Qudrat-i-Khuda, H. N. De and Nur Mohammed Khan, Pakistan J. Sci. Ind. Research, **3**, 10 (1960).