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BIOCHEMICAL ASPECTS OF THE DEVELOPING AND RIPENING BANANA

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Key *Word Index*—*Musa cavendishii*; Musaceae; *banana fruit development and ripening*; *pseudostem*; transaminase; aldolase; protein; phenolics; chlorophyll; starch.

Abstract—The peel and pulp of the banana fruit and the pseudostem were examined for glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and aldolase activities and protein, phenolics, chlorophyll and starch. The peel-pulp ratio at various stages of fruit development on the plant and in detached fruits showing incipient ripening were used as an index of the physiological age of the fruit. The enzymes exhibited maximum activity at a stage corresponding to the initiation of the climacteric. GPT level at this stage was higher than that of GOT. An initial increase in the protein content was followed by a decline in both peel and pulp, the level reaching a minimum in climacteric fruits. Astringency, measured in terms of total phenolics, decreased with development; in mature fruits, peel contained 4–5× as much phenolics as pulp. Chlorophyll in mature fruits was 10× higher than in young fruits and decreased in ripe fruits. The onset of ripening was attended with a pronounced decrease in the starch. The various analyses were carried out also on the pseudostem removed from the plant soon after flower formation.

INTRODUCTION

THE BIOCHEMICAL constituents of the banana fruit which have been investigated include carbohydrates,[1–3] protein,[4,5] pigments and phenolic compounds,[6–8] vitamins,[9] lipids,[10] organic acids[11] and various enzymes.[12–14] Most of the existing reports are on detached bunches, there being no systematic study on the biochemistry and metabolism of fruits during development on the plant.

The pulp of the banana fruit has been more thoroughly examined than the skin, which is generally discarded. It might be expected that the peel, on account of its photosynthetic ability, contributes significantly to the overall metabolism of the fruit. The pseudostem, which bears the inflorescence, has hardly been studied in relation to the biochemistry of the banana fruit. It therefore appeared of interest to conduct studies simultaneously on

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peel and pulp of the fruit and on the pseudostem. The two key enzymes involved in transamination, glutamate-oxaloacetate transaminase (E.C. 2.6.1.1, L-aspartate: 2-oxoglutarate amino transferase) and glutamate-pyruvate transaminase (E.C. 2.6.1.2, L-alanine: 2-oxoglutarate amino transferase) and the glycolytic enzyme aldolase (E.C. 4.1.2.b, fructose-1,6-diphosphate D-glyceraldehyde-3-phosphate-lyase) were studied during different stages of fruit development on the plant and in harvested fruits showing incipient ripening. Simultaneously, protein, phenolics, chlorophyll and starch were determined.

RESULTS

GOT, GPT and aldolase activities are expressed as units per mg protein of the tissue. Dry solids have been reported as a percentage and other analytical data as mg/g dry wt of tissue (Table 1).

Enzyme activity

GOT and GPT. In the pulp, there was a progressive increase in GOT and GPT activities as the young fruits matured on the plant. Both enzymes were maximally active at a period corresponding to the climacteric stage. In young fruits, GOT activity exceeded GPT activity, but in mature and ripe fruits this pattern was reversed. The two activities in the peel followed a pattern similar to that in pulp but at a somewhat lower level. In the peel of young unripe fruits, the two enzymes were equally active while in the full grown and ripe fruits GPT activity exceeded GOT, the difference being most conspicuous in climacteric fruits.

In the pseudostem, GPT was twice as active as the GOT system; the activity of the latter was 1·5× that in pulp and 4·5× that in the peel, while GPT activity was 6 and 10× the activity respectively in pulp and peel of the youngest banana fruit.

Aldolase

The growth of the banana fruit was attended with an initial drop in aldolase activity in both the pulp and peel, followed by an increase until in the fully mature fruit the enzyme was over 2× as active as in the initial stage. The activity was unaltered with the onset of ripening. At all stages of growth, the activity in peel was less than in the pulp. Aldolase activity in the pseudostem was twice as active as in peel and 40% higher than in pulp of young fruit.

Dry solids

As the banana fruit matured on the plant, there was an increase in the per cent of dry solids in the pulp. In the fully mature fruit, the per cent dry solids in the pulp reached 30, being almost 4× that in the youngest fruit. In fruits ripening incipiently off the plant, there was about 17% diminution in the per cent of pulp dry solids. There was a gradual increase in the dry weight of the peel in the growing fruit, levelling out when maturity was attained, followed then by a 2× rise. In the initial stage of fruit setting, the dry weight percentage was the same in peel and pulp, but in the fully mature fruit the per cent dry matter was about one-third that in pulp. The dry solids of the pseudostem constituted 64% of the concentration in peel or pulp of the young banana fruit.

Protein

Protein concentration in the pulp was maximum in the earlier stages of fruit development. Subsequently, there was a pronounced decrease with increasing maturity and in the full

TABLE 1. CHANGES IN GOT, GPT AND ALDOLASE ACTIVITIES AND PROTEIN, PHENOLICS, CHLOROPHYLL AND STARCH IN PULP AND PEEL OF THE BANANA FRUIT DURING DEVELOPMENT AND IN THE PSEUDOSTEM

Peel/ pulp ratio	Activity, units/mg protein			Dry wt (%)	mg/g dry wt			
	GOT	GPT	Aldolase		Protein	Phenolics	Chlorophyll	Starch
				Pulp				
4·42	6·7	4·2	198	7·1	38·9	25·6		91·5
3·85	7·0	5·5	139	7·3	58·2	18·1		169·8
3·30	6·7	5·6	110	9·6	55·8	11·3		132·0
2·90	9·4	5·3	117	9·0	59·2	16·2		185·5
1·57	9·7	3·1	138	9·1	54·9	12·5		382·2
1·48	10·5	8·5	142	13·4	36·6	6·2		520·1
1·28	9·8	8·5	150	13·7	35·5	7·5		530·0
0·54	9·4	12·2	155	23·7	20·7	3·1		764·5
0·30	13·7	19·2	198	28·7	15·3	2·6		880·8
0·28	18·3	24·2	380	25·5	9·0	2·2		402·3
0·22	24·8	30·5	432	29·9	7·0	2·5		374·9
0·20	29·6	49·7	447	25·7	5·0	1·2		16·3
0·19	27·4	52·0	450	25·0	5·6	1·5		11·2
				Peel				
4·42	2·62	2·62	135	7·1	59·3	30·6	0·085	94·8
3·85	2·22	2·40	117	7·1	64·6	22·5	0·253	164·9
3·30	3·0	3·0	90	8·1	72·8	22·8	0·296	134·7
2·90	3·3	3·6	75	7·2	97·2	21·7	0·333	152·4
1·57	5·4	5·1	108	8·4	60·5	20·9	0·190	166·3
1·48	5·3	4·8	109	9·2	54·3	12·5	0·920	169·0
1·28	5·4	5·7	111	9·1	58·7	12·3	0·800	285·2
0·54	4·2	9·0	103	10·5	47·6	10·8	0·810	206·9
0·30	6·4	10·3	121	9·1	49·1	13·1	0·890	297·7
0·28	6·6	7·3	130	9·3	44·1	12·6	0·709	106·7
0·22	11·1	12·9	172	9·3	30·8	10·2	0·860	106·0
0·20	15·3	33·3	225	16·0	8·3	7·0	0·137	31·4
0·19	16·3	35·6	236	17·7	7·5	7·3	0·130	23·4
Pseudo-stem	11·2	25·6	277	4·6	17·2	11·8		36·6

The first hand to emerge from the inflorescence was used for the various analyses, unless otherwise mentioned. The hands chosen ranged from 7–100 days on the plant. The peel–pulp ratio was determined on dried samples. The stages corresponding to ratios of 4·42–1·28 represent young unripe fruits, the maximum ratio corresponding to the youngest fruit. The four ratios which follow (0·54–0·22) represent mature fruits, full maturity being attained at a ratio of 0·22. At this stage, two bunches were harvested from plants in which the first hand had been removed and allowed to ripen. The two ratios viz 0·20 and 0·19 refer to the stage of incipient ripening of the two bunches or the climacteric. The pseudostem was collected from plants in which the first hand had emerged. Values for pseudostem are the mean of two separate determinations. Protein and chlorophyll were estimated in 10% (w/v) homogenates prepared in 0·05 M- phosphate buffer, pH 7·5 and supplemented with 0·02 M-freshly neutralized cysteine hydrochloride.

grown banana fruit the concentration was only one-eighth of the maximal value. The maximal value of protein in the peel, at a peel–pulp ratio of 2·90, exceeded the protein concentration in pulp by about 64%. Protein increased initially, reached a maximum and thereafter declined. The protein in the fully mature banana peel was only about one-third the maximal value attained in earlier stages of fruit development. Protein concentration in peel was higher than in pulp at all stages of growth of the fruit. Protein of the pseudostem corresponded to 29 and 44% of the concentration respectively in peel and pulp of the young fruit.

Phenolics

Phenolics occurred in the highest concentration in the pulp of youngest fruit. As the

fruit matured there was diminution in the phenolics, the concentration being reduced to one-tenth in the fully grown fruit. The concentration diminished further by 40% in the climacteric stage. As in pulp, the phenolics in the peel showed a decrease in concentration with progressive maturation. In the fully grown fruit, it was a third at the initial stage of fruit formation. At all stages of growth, the concentration of phenolics in peel was higher than in pulp. The phenolics concentration in the pseudostem was considerably lower than in the young banana fruit, representing 38 and 46% the concentration respectively in peel and pulp.

Chlorophyll

From a low initial value, total chlorophyll in peel showed a steady increase and more or less levelled off at the high concentration. With the onset of ripening, there was a marked drop in the chlorophyll concentration, coinciding with the yellowing of the skin.

Starch

In the earliest stage of fruit formation, starch constituted 9% of the dry wt of the pulp, whereas in the mature fruit (peel–pulp ratio 0:30) it comprised 88%. Evidently, as the banana fruit developed on the plant, massive synthesis of starch occurred. Just preceding the climacteric, hydrolysis of starch was initiated and only about one-half remained. In climacteric fruits starch almost disappeared. In the earliest stage of growth, starch concentration in the peel was the same as in the pulp. With growth and maturation of the fruit there was a progressive increase in starch concentration, the maximum value attained being about a third that in the pulp. Subsequently, there was a steep fall. In the climacteric stage starch represented only 2–3% of the dry wt of peel. Starch constituted only 3–4% of the dry weight of the pseudostem, representing about a third of that in pulp and peel.

DISCUSSION

Although transamination reactions have been widely studied in fruits, their relation to fruit ripening has not been thoroughly investigated.[15–17] In tomatoes, there is a fall in cytoplasmic GOT during ripening while mitochondrial activity reaches its peak in green-orange fruit.[18] In *M. cavendishii*, both pulp and peel tissue exhibited significant transaminase activity which increased progressively with fruit development, maximum activity being attained when the fruit exhibited incipient ripening. At this stage the GPT system was more active than GOT system in both the skin and pulp. Since, at the same time, there is a considerable decrease in the protein it would appear that amino acids give rise to intermediate(s) of the Krebs cycle and undergo oxidation thereby providing an important link between carbohydrate and protein metabolism. Active mitochondria have been isolated capable of oxidizing Krebs cycle acids and pyruvate.[19] Steward *et al.*,[20] in Gros Michel bananas, showed a depletion during growth of alanine, aspartic acid and some other amino acids and also α-ketoglutaric acid and pyruvic acid.

Young[21] claimed that aldolase activity in pre- and post-climacteric banana tissue

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remained constant if precautionary measures were taken against interference by endogenous tannins. In the present experiments, however, aldolase activity decreased gradually in the initial stages of fruit development, then increased and reached a maximum at incipient ripening. This was in agreement with the findings of Tager and Biale[22] who attributed the increase to a shift from the pentose phosphate pathway to the glycolytic pathway. Such an increase was to be expected in view of the need to provide additional pyruvate from carbohydrate sources for oxidation in mitochondria. There is evidence for increased mitochondrial activity at the climacteric.[23] That aldolase activity attained its peak in peel tissue when chlorophyll content was minimum is significant. In developing pea leaves, also, the enzyme was found to be present in relatively large amounts when the photosynthetic rate was very low.[24]

The observed pattern of the steady increase in dry solids content up to full maturity was in agreement with the report by Barnell.[25] The decline in protein concentration towards the later stages of fruit development observed in the present experiments was in conformity with the observation of Sacher,[4] though at variance from that of Brady *et al.*[26]

Astringency in unripe fruits caused by leucoanthocyanins disappeared during ripening on the tree or on storage.[27] In *M. cavendishii*, at the climacteric stage, only 4–5% of the initial content of phenolics was left behind. Whether phenolics, in the banana fruit, serve as protective agents and as a source of metabolisable reserve is uncertain. Recent studies point to a role of phenolic compounds in the metabolic regulation of redox potentials.[28]

In Gros Michel bananas starch accumulated for about 100 days and then was extensively depleted from 110–130 days.[25] An analogous pattern was encountered in the present study with *M. cavendishii*. This is to be expected since starch provides the main source of energy for respiration. In the peel, apparently, starch accumulation results from photosynthetic carbon assimilation.

The several-fold higher transaminase- and aldolase-activities in the pseudostem tissue, in comparison with the fingers in the hand which had formed at the time the plant was cut, taken together with the significant amount of protein and the large mass of the tissue, was indicative of an active role played by it in the development of the banana fruit. Besides serving as a channel for nutrients, it is also possible that intermediates elaborated in the bunch are, in part, further metabolized in the pseudostem or even in the rootstock. Apparently, the peel also contributes to the development of the pulp and is an actively metabolizing tissue. Until the hand was 65 days old, the (dry) mass of peel exceeded that of pulp. The peel tissue is known to possess also functionally active mitochondria.[29]

EXPERIMENTAL

Plant tissue and sampling. The fruit of the dwarf banana plant *Musa cavendishii*, Lambert ex Paxt (edible variety), was used in the study.

After removing the peel, the flesh of each fruit was sliced longitudinally and the central core with embedded seeds discarded. The pseudostem was freed of surrounding leaf sheaths and cut into small pieces as with peel and pulp tissue. Small random lots were weighed as required.

Preparation of homogenates and media employed. For transaminase, a 20% homogenate was prepared in 0·05 M-phosphate buffer, pH 7·5; 0·02 M-EDTA, pH 7·0 and 0·02 M-freshly neutralized cysteine HCl and supplemented

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with 1% Triton X-100 and 1% polyvinylpyrrolidone (av MW 10000). For the assay of aldolase, the tissue (10%) was ground in 0·05 M-Tris–HCl, pH 8·0; 0·0025 M-freshly neutralized $Na_2S_2O_5$ for pulp and 0·005 M for peel; 0·02 M-EDTA, pH 7·0 and 1% Triton X-100. Homogenization was effected in a chilled Waring blendor, operated at full speed for 1 min. The final volume was made up after filtration through two folds of muslin.

The incorporation of PVP and cysteine in the medium for transaminase and $Na_2S_2O_5$ in the medium for aldolase activity favoured maximum activity by suppressing the inhibitory effect of phenolics. The optimum concentrations remained unaltered when an early stage of development and a late stage of development of the fruit were tested.

Enzyme assay. The standard incubation mixture for GOT and GPT and color development was as described by Tonhazy *et al.*[29] with minor modifications. 1 unit of enzyme activity is that which causes the formation of 1 μmole of pyruvate in 30 min at 37°. The assay system for aldolase in a totol vol. of 1·0 ml consisted of: 0·10 M-Tris-HCl buffer, pH 8·6, 0·4 ml; 0·22 M-hydrazine, pH 8·6, 0·10 ml; enzyme prep., 0·05 ml (10% w/v); 0·01 M fructose-1,6-diphosphate, pH 7·0, 0·2 ml and 0·25 ml H_2O. The reaction was initiated by the addition of substrate in the experimental tubes and terminated by the addition of 2·0 ml 10% TCA. Colour was developed according to the modified Sibley and Lehninger's[30] method and read at 540 nm. One unit of enzyme is that giving a difference of 100 on Klett Summerson's colorimeter (1 cm cell) at 30° for 45 min and pH 8·6.

Other estimations. Protein was estimated according to the method of Lowry *et al.*[31] Chlorophyll was determined spectrophotometrically, employing the formula given by Arnon.[32] Total phenolic content was determined in ethanol extracts as described by Goldstein and Swain,[33] using tannic acid as standard. Starch was estimated in dry samples of residue obtained after ethanol extraction as described by Pucher *et al.*[34] with modifications.[35]

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