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CARBOHYDRATE METABOLISM IN RIPENING BANANA FRUIT

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Key Word Index—*Musa sapientum*; Musaceae; banana; softening; carbohydrates; carbohydrate hydrolases; ^{14}C incoporation.

Abstract—A comprehensive picture of changes in carbohydrates, carbohydrate hydrolases, cell structure and texture in banana fruit during ripening is described. The insoluble carbohydrates were separated into seven fractions based on differential solubility. Their profiles and composition were followed as hydrolysis products. The starch and pectic fractions decreased considerably in ripe banana pulp. The significant decrease in glucose concentration in the hydrolysates of cold and hot water-soluble polysaccharides and that of xylose in the hydrolysates of hemicellulosic fractions (Hem A and B), indicated a pronounced glucan and xylan degradation during ripening. Some loss of mannose from the Hem "B" fraction at the ripe stage was also noticed. The various carbohydrate hydrolases, *viz.*, polygalacturonase, pectin methyl esterase, xylanase, laminarinase, α-mannosidase, β-galactosidase, amylase, cellulase and hemicellulase registered a general increase in their activities. Low levels of endo-β-mannanase and galactanase activities could be detected only at the climacteric stage of ripening. More than 80% of the radio-activity of [^{14}C] starch was incorporated into soluble sugars, *viz.*, glucose, fructose and sucrose, indicating active sugar interconversions. The total content of these soluble sugars increased from 1.8 to 19%, with a concomitant decrease in starch content during ripening. Microscopically, loss of cell wall integrity, cell wall thinning, increased intercellular spaces, loosening of cells and disappearance of starch granules were evident. In banana, it appears that pectinase may play a more dominant role in softening than cellulase. Amylase, xylanase and laminarinase may also contribute to loosening of cellular structures.

INTRODUCTION

Fruit ripening is a genetically programmed, highly co-ordinated process of organ transformation from unripe to ripe stage [1], to yield an attractive edible fruit with an optimum blend of colour, taste, aroma and texture [2]. From an economic standpoint, textural change is the most crucial of all, as it directly affects the shelf-life of the fruit and its keeping quality [3, 4]. The importance of understanding the softening process and its implications are emphasised in a recent review on carbohydrate solubilization in fruit ripening [5]. Modification of fruit ripening by suppressing expression of specific enzymes has been extensively studied and demonstrated in tomatoes [6, 7]. Banana, with its innumerable varieties, is a tropical fruit of commercial significance. The physical parameter of textural firmness in relation to chemical composition was very recently reported in "desert" and "cooking" bananas [8]. The present study aims to understand, in biochemical terms, the textural softening of this fruit, bringing all the related parameters into focus, *viz.*, physical, biochemical and microscopical. This approach was necessary for a comprehensive analysis in banana. Recently, we reported a similar study on capsicum fruit ripening [9].

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RESULTS AND DISCUSSION

Fruit softening

Softening in the pulp during ripening was significant as measured by the compression test on the pulp which decreased from 314 to 15 N mm^{-2} from the raw to the ripe stage. The shear force decreased only to the extent of five-fold (from 133 to 27 N mm^{-2}), indicating a much lesser degree of textural softening in the peel.

Major carbohydrate polymers

Starch (~18%) had almost disappeared at the ripe stage. Total hemicellulose content lowered considerably from 2.4% to 0.9% during ripening, whereas pectin decreased from 1.1% to 0.8%. There was no apparent change in cellulose.

Table 1. Sugar composition of fractionated polysaccharides of banana after hydrolysis at raw and ripe stages*

Alcohol- insoluble fraction**		Sugars detected (mg/g $^{-1}$ fr. w)						
		Total	Rha	Ara	Xyl	Man	Gal	Glc
CWS	Unripe	8.0↓	0.003	0.023	0.012	0.06	Tr	7.8↓
	Ripe	5.0	Tr	Tr	4.9↑	Tr	0.05	Tr
HWS	Unripe	3.0	Tr	Tr	0.05	Tr	Tr	2.9
	Ripe	5.0	1.50↑	1.51↑	0.90↑	0.30	0.40	Tr↑
PEC	Unripe	12.0↓	0.2↓	0.06↓	0.9↓	Tr	Tr	10.2↓
	Ripe	6.5	0.06	0.02	0.05	Tr	Tr	6.2
Hem-A	Unripe	8.0↓	Tr	Tr	7.9↓	Tr	Tr	Tr
	Ripe	3.0	0.6	0.12	2.2	Tr	Tr	Tr
Hem-B	Unripe	7.0	Tr	Tr	5.6↓	1.3	Tr	Tr
	Ripe	5.0	0.01	0.001	0.25	0.3	0.09	4.25↑
Ce/Li	Unripe	6.0	0.03	0.15	0.15	0.07	0.19	4.9
	Ripe	5.8	Tr	0.29	0.28	Tr	Tr	5.05

* Unripe 1 g fruit = 220 mg ripe 1 g fruit = 40 mg d.w.

** Starch completely removed by glucoamylase digestion.

CWS: Cold water solubles, HWS: Hot water solubles, PEC: Pectic fractions, Hem - A & B: Hemicellulose A & B, Ce/Li: Insoluble cellulose & Lignin. Tr: Less than 1 μg.

Arrows indicate significant changes. ↓: Decrease in ripe stage. ↑: Insrease in ripe stage.

Values are means of 3 analyses.

Soluble sugars

The increase in total alcohol-soluble sugar concentration from unripe (1.8%) to ripe (18.6%) was 10-fold. HPLC analysis of the individual sugars revealed the presence of glu, fru, suc, mal and small concentrations of rha at the ripe stage. In ripe pulp, fru was the highest in concentration followed by glu and suc, the values being 7.46, 6.85 and 3.70 gm%, respectively. Mal and rha were not detectable at the raw stage.

The conversion of [^{14}C] starch into soluble sugar fractions in the raw and ripe banana were monitored. Interconversions among soluble sugars from glu to fru and glu to suc were evident from [^{14}C] starch incorporation into these sugars. The rates of these conversions were much higher at the ripe stage.

Alcohol insoluble carbohydrates

The alcohol-insoluble residue (total) was subjected to differential extractions to yield various fractions (Table 1) and their hydrolytic profiles were analysed. From the GLC analysis of the constituent sugars, it could be inferred that there was a complete disappearance of glu in the hydrolysates of the cold water-soluble (CWS) and hot water-soluble (HWS) fractions as a result of ripening, which was indicative of glucan/glucomannan type degradation. A significant reduction in xyl from the hemicellulose (Hem) "A" and Hem "B" fractions suggested xylan-or xyloglucan-type degradation. The reduced yields of the pectin fraction was not truly reflected in the sugar profiles of the hydrolysates, as the major pectic component, that is galacturonic acid, does not show up in this sugar profile. However, the total free uronic acid increased by six-fold during ripening, i.e., 30 mg% to 180 mg% from the raw to the ripe stage. A decrease in the rha concentrations in the hydrolysate of the pectic fraction was also noted. (Acidic pectin is made up of rhamno-galacturonan in the ratio of 1:43). There was negligible cellulose solubilization, as evidenced by unchanged amounts of glu in the hydrolysates of the cellulose fraction.

Whilst mainly a degradative process, the CWS and HWS fractions in ripe banana yielded, on hydrolysis, higher amounts of xyl. Similarly, there was increased contents of rha and ara in the HWS fraction at the ripe stage. The glu concentration in Hem "B" of ripe banana was also higher. These observations indicated the formation of hemicellulosic-type polymers during ripening, probably at the expense of hydrolysed sugars *in vivo*.

Carbohydrate hydrolases

Activities of the carbohydrate hydrolases involved in carbohydrate hydrolysis *in vivo* are depicted in Fig. 1 A and B. Among the pectinases, PG activity remained almost constant, while PME showed a climacteric peak, registering a six-fold increase during ripening. Cellulase and hemicellulase activities increased until the 3rd day, followed by a small decrease. Amylase and xylanase showed a steady increase in activity, while α-mannosidase and laminarinase (β-1,3 glucanase) exhibited a peak in their activities around climacteric. β-Galactosidase activity always remained low during ripening. Unlike other enzymes, the activity of xylanase was quite high in the

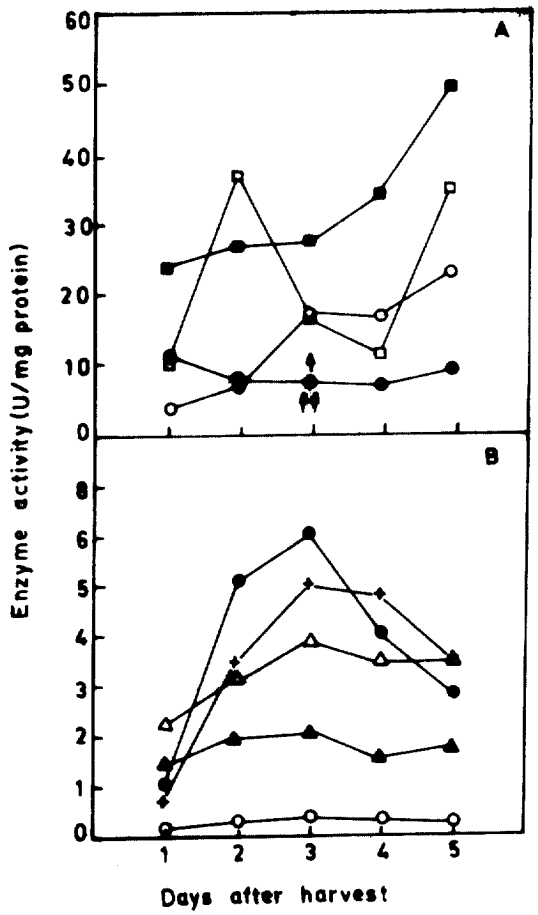

Fig. 1. Specific activities of carbohydrate hydrolases extracted from banana pulp at different stages of ripening.(A) Xylanase (-■-), laminarinase (-□-), PG (-●-), amylase (-○-), mannanase (↑) and galactanase (↑↑).(B) PME (-●-), α-mannosidase (-×-), β-galactosidase (-○-), hemicellulase (-△-) and cellulase (-▽-).Values are means of three individual fruits and the SD was within 7%.

unripe stage and increased further during ripening. Interestingly, xylanase activity in banana fruit appears to be higher than that in other fruits, *viz.*, mango, papaya and capsicum (data not shown). Low endo-mannanase and galactanase activity could be detected only at the climacteric stage.

Microscopy

At the raw stage (Fig. 2 A and B), the epicarp clearly revealed an outer cuticle and epidermis, while the hypodermis contained parenchymatous cells (Fig. 2A). The inner mesocarp (Fig. 2B) showed scattered patches of starch granules of a larger size and number compared with those in the region near the epicarp. At the ripe stage (Fig. 2 C and D), loss of cell integrity and cell wall degeneration, with thinning of the cell wall was evident. Increased intercellular spaces, loosening of cells and disappearance of starch granules were obvious. Cell compactness was also lost. Our study identifies the crucial carbohydrate polymers and the key enzymes contributing to textural softening in banana. This could form a basis for manipulating fruit ripening at the textural level. The extent of tissue softening, loss of cellular intactness, extensive hydrolysis of some carbohydrate polymers and increased activity of some carbohydrate hydrolases during ripening show a clear correlation between one another. Much of the soluble glu accumulated in ripe pulp is probably due to starch hydrolysis, as also corroborated by the concomitant disappearance of starch in the ripe fruit and by the [^{14}C] incorporation into the sugar fractions from starch. Degradation of the glucan in the CWS fraction may also contribute to increased free glu. Increase in fru and suc at the ripe stage could be due to glu interconversions and suc synthetic enzymes, which are well documented in plant systems [10].

The degradation of pectic substances, xylan from the hemicellulose fraction and glucan from the CWS and HWS fractions was considerable at ripe stage, as indicated by the hydrolytic profiles. Ripening brought a general increase in the enzymes involved in polysaccharide hydrolysis. From these observations on hydrolytic profiles of carbohydrates and the corresponding enzyme activities at five different stages of ripening, it is expected that amylase, pectinases, xylanase and laminarinase in banana pulp may play a dominant role in loosening of cell structure and tissue softening.

In tomato, the major enzymes affecting fruit firmness are reported to be PG and PME [11, 12] and in avocado, cellulase [13, 14]. In the former, increased firmness, increased total soluble solids and longer shelf-life is clearly demonstrated by suppressing individually the expression of PG, PME and ethylene-forming enzymes, respectively [11, 12, 15, 16]. The most prominent enzymes of banana in this respect appear to be pectinases, amylase, xylanase and laminarinase.

EXPERIMENTAL

Freshly harvested mature raw fruits (*Musa sapientum* cv. Robusta), were brought from fruit dealers. Fruits obtained immediately after harvest denoted extreme unripe stage (raw). Subsequent stages of ripening were picked from fruits stored at $27 \pm 2^{\circ}$. Fruits from five different stages representing consecutive days after harvest were used for enzyme analysis.

Texture measurements

Texture measurements were conducted using a texture analyzer (High Wycombe model 4301 texture analyser, Instron Ltd.,). Shear resistance by the intact fruit was measured using a 10 kg load cell and a stroke speed of 100 mm min^{-1} For compression tests, uniform cylindrical samples of 2 cm length were cut longi-

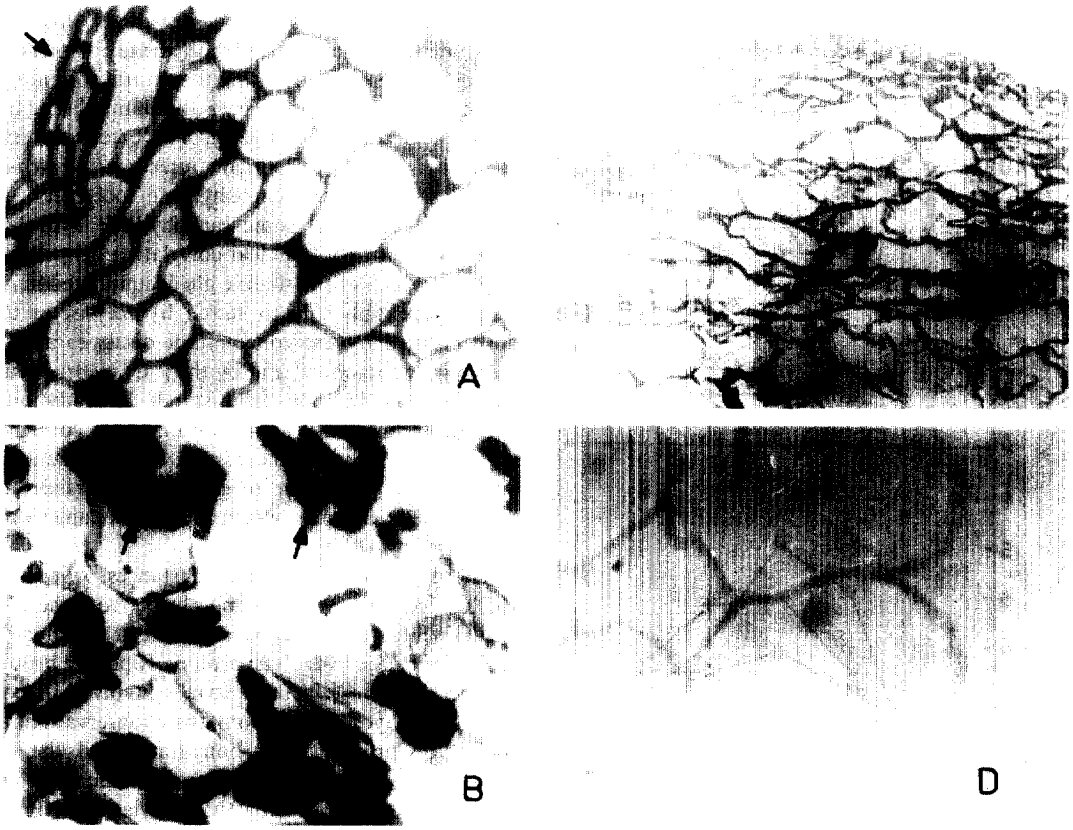

Fig. 2. Photomicrographs of banana showing cellular details at raw and ripe stages. (A and B) Unripe stage. A, epicarp with intact cuticle (arrow) and hypodermis with intact parenchymatous cells. B, endocarp with dense starch granules (arrow). (C and D) Ripe stage. C, degenerating epicarp and mesocarp with loosely arranged cells. D, endocarp with collapsed cell walls and complete disappearance of starch.

tudinally from the pulp with the help of a cork borer. The apparent strength of the sample was calculated by dividing the maximum force required for 50% compression by the cross-sectional area of the sample and was recorded as N mm $^{-2}$.

Carbohydrate analysis

Endogenous metabolic activity of the fruit was arrested by quickly fixing a known wt. of tissue in *ca* 15 vols of 80% EtOH and the sample was blended in a homogenizer and filtered through four layers of nylon cloth. The residue was re-extracted and the combined extracts pooled, concd by flash evap at 40°, centrifuged and washed with petrol. The aq. layer, after passing through Dowex-50W (H^+) and Dowex-1 (OH^-) resins [17], was collected and suitably concd for PC, HPLC and colorimetric determinations.

Reducing and total sugars were estimated by the Nelson–Somogyi [18] and PHOH-H_2SO_4 [19] methods, respectively. For qualitative profiles and radioactive determination of individual sugars, descending PC was carried out on Whatman No. 1 using *n*-PrOH-EtOH-H_2O- (7:1:2) [20]. Sugars were detected by aniline phthalate and urea hydrochloride spray reagents. Soluble sugars were quantitated by HPLC on an aminopropyl column (Shimadzu) with a RI detector using MeCN-H_2O (7:3) at a flow rate of 1 ml min^{-1}.

The EtOH-insol. residue was sequentially extracted with (1) cold H_2O for CWS, (2) hot H_2O for HWS, (3) 0.5% EDTA for pectins, (4) 4N KOH for Hem "A" and (5) Hem "B" (The 4N KOH extract was adjusted to pH 4.5; the resultant ppt. was designated Hem "A". The supernatant was pptd with 3 vols of EtOH to give Hem "B") and finally to yield the (6) non-extractable cellulose-lignin fr.[20]. All the frs were pptd with 80% EtOH, recovered, washed with petrol to remove pigments and lipids, and finally washed with Me_2CO to make it moisture free. Dry residue (10 mg) was hydrolysed using H_2SO_4 (72 gl^{-1}) and the derived neutral sugars were quantitated and identified as alditol acetates by GC [21] on an OV-225 column and a FID detector (N_2 flow-rate 30 ml min^{-1}, column temp 200°, inj. temp 220°, detector temp. 250°). Starch was estimated by glucoamylase digestion followed by

glucose determination using the dinitrosalicylic acid method [22]. All values mentioned in the text are on fr. wt basis.

Radioactive studies

Labelled compound was suitably diluted with 0.4 M mannitol sol to give 1.8 μCi in 0.05 ml which was injected directly into the pulp portion through a syringe by vacuum infiltration [23]. The amount of the labelled compound entering into each fruit was calculated by subtracting the counts of syringe wash from the total counts taken initially. Fruits injected with labelled starch were transferred to desiccators individually fitted with an inlet for air [24] and were kept for respiration for 8 hr. The pulp portion of the fruit was homogenised after 8 hr. Known aliquots were fixed in 70% EtOH for EtOH-sol. sugars. Extraction and purification of sugars was as described above. A known aliquot of the sugar fr was analysed for radioactivity. Liquid scintillation counting was done in Bray's cocktail. Counts are expressed as % incorporation [24].

Enzyme extraction and assay

Me_2CO powders from banana pulp were prepared in triplicate from the five different stages of ripening. They were (1 g each) extracted in 10 ml extraction buffer, homogenized, clarified and dialysed. Buffers used for extraction and assay of various carbohydrate hydrolases and expression of the enzyme activity units are described in detail in ref.[9]. PME activity was monitored by the decrease in pH upon demethylation. Mannosidase and galactosidase were assayed by following the release of PNP, while the rest of the enzymes were assayed by measuring the reducing group.

Microscopy

Preparation of banana tissue, the dehydration process, tissue embedding, sectioning and staining by PAS (periodic acid Schiff's base) were done following standard procedures [25].

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