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ENZYMES OF THE BANANA PLANT: OPTIMUM CONDITIONS FOR EXTRACTION

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Abstract—The activities of starch phosphorylase, β-amylase, phosphohexoisomerase, acid and alkaline invertase, sucrose synthetase, sucrose phosphate synthetase and acid and alkaline phosphatase were determined in various parts of the banana plant, using homogenates prepared in media supplemented with polyvinylpyrrolidone or Triton X-100. The results indicated that the supplement of choice depended on the enzyme and the tissue under study.

INTRODUCTION

YOUNG[1] reported that in banana fruits a leucoanthocyanin-type tannin is concentrated in the latex cells. Tannin was shown to have an inhibitory action on banana aldolase,[1] amylase[2] and phenol oxidase.[3] Khanna *et al.*[4] showed that starch phosphorylase activity in the leaves of *Dendrophthoe falcata* was powerfully inhibited when cell-free preparations were made in ordinary media. The inhibition could be overcome by removal of endogenous phenolics. The experiments reported in the present communication were designed to test the effects of supplementation of the dispersion medium with polyvinylpyrrolidone (PVP) or Triton X-100 on certain enzymes extracted from the banana plant. These reagents were chosen to 'fix' or 'sequester' the phenolics or to rupture any complexes formed between phenolics and protein. In view of the likely variations in the content of the enzyme-inhibitory substances in different parts of the banana plant, the efficacy of the above supplements was tested with tissues from all parts of the banana plant.

RESULTS

Distribution of Phenolics in Different Parts of the Banana Plant

The results are recorded in Table 1. Expressed on a fresh weight basis, phenolics were concentrated largely in the leaves, followed by the fruits and the rootstock. The pseudostem contained the lowest concentration of phenolics.

Enzyme Activities in Different Parts of the Banana Plant

Leaves

No alkaline phosphatase (E.C. 3.1.3.11, orthophosphoric monoester phosphohydrolase) activity was demonstrable in banana leaves when the homogenate was prepared in the basal medium or the medium supplemented with Triton X-100. Inclusion of PVP in the homogenizing medium was essential to demonstrate the enzyme activity.

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[3] A. N. BADRAN and D. E. JONES, *Nature, Lond.* **206**, 622 (1965).
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TABLE 1. DISTRIBUTION OF PHENOLICS IN THE BANANA PLANT

Tissue	Total phenolics* (mg/g fresh wt.)
Leaf	1·80
Leaf-sheath	0·48
Rootstock	0·95
Lower pseudostem	0·55
Upper pseudostem	0·33
Fruit	1·50

* Total phenolics have been expressed in terms of tannic acid equivalent.

PVP supplementation led to 2-fold increase of sucrose synthetase (E.C. 2.4.1.13, UDP-glucose: D-fructose 2-glucosyltransferase), 2·4-fold of alkaline invertase (E.C. 3.2.1.26, β-fructofuranoside-fructohydrolase) and 3·8-fold of starch phosphorylase (E.C. 2.4.1.1, α-1,4 glucan: orthophosphate glucosyltransferase) activities (Table 2). However, acid invertase (E.C. 3.2.1.26, β-fructofuranoside fructohydrolase) activity was lost when the homogenate was prepared in PVP-supplemented medium. Maximum activities of acid invertase, acid phosphatase (E.C. 3.1.3.2, orthophosphoric monoester phosphohydrolase) and phospho-hexoisomerase (E.C. 5.3.1.9, D-glucose 6-phosphate ketol-isomerase) were obtained when the basal homogenizing medium was supplemented with Triton X-100; the treatment resulted in 17, 1·8 and 1·4-fold increase in activities respectively. The activity of sucrose phosphate synthetase (E.C. 2.4.1.14, UDP-glucose: D-fructose 6-phosphate 2-glucosyl-transferase) was highest in the basal medium and PVP or Triton X-100 incorporation caused 70 and 85% inhibition of the enzyme activity.

TABLE 2. ENZYME ACTIVITIES IN BANANA LEAVES

Enzyme	Enzyme activity, units/g fresh wt.		
	Basal medium	Basal medium + 1% PVP	Basal medium + 1% Triton X-100
Sucrose synthetase	2·60	5·20	4·85
Sucrose phosphate synthetase	13·40	4·40	2·20
Acid invertase	0·28	Nil	4·75
Alkaline invertase	2·18	5·20	4·75
β-Amylase	Nil	Nil	Nil
Starch phosphorylase	2·30	8·64	4·61
Acid phosphatase	3·68	5·53	6·45
Alkaline phosphatase	Nil	1·84	Nil
Phosphohexoisomerase	20·31	21·42	26·10

The basal medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine and 0·01 M EDTA.

Leaf-sheaths

Sucrose phosphate synthetase, acid phosphatase and phosphohexoisomerase could not be demonstrated in banana leaf-sheaths, unless PVP was incorporated in the basal medium.

The inclusion of PVP in the medium led to enhancement in the activities of β-amylase (E.C. 3.2.1.2, α-glucan maltohydrolase) and starch phosphorylase by 1·8 and 4-fold respectively (Table 3). Sucrose synthetase activity was, however, lost in PVP-supplemented homogenate.

TABLE 3. ENZYME ACTIVITIES IN BANANA LEAF-SHEATH

Enzyme	Enzyme activity, units/g fresh wt.		
	Basal medium	Basal medium + 1% PVP	Basal medium + 1% Triton X-100
Sucrose synthetase	3·35	Nil	Nil
Sucrose phosphate synthetase	Nil	3·80	Nil
Acid invertase	Nil	Nil	1·87
Alkaline invertase	3·78	5·65	11·30
β-Amylase	1·25	2·25	Nil
Starch phosphorylase	0·58	2·31	1·73
Acid phosphatase	Nil	2·77	1·84
Alkaline phosphatase	Nil	Nil	Nil
Phosphohexoisomerase	Nil	12·80	12·80

The basal medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine and 0·01 M EDTA.

Maximum alkaline invertase was demonstrated in the medium containing Triton X-100, wherein 3-fold increase in activity was obtained compared to the unsupplemented medium. Acid invertase activity was demonstrable only when the homogenization of the tissue was carried out in Triton X-100 supplemented medium. In contrast, sucrose synthetase, sucrose phosphate synthetase and β-amylase activities were completely lost on supplementation of the medium with Triton X-100.

Rootstock

Acid phosphatase activity could be demonstrated in banana rootstock only when the basal homogenizing medium was supplemented with either PVP or Triton X-100. Maximum activities of sucrose synthetase, alkaline invertase and starch phosphorylase were demonstrated in the homogenate supplemented with PVP; the treatment resulted in 2-fold increase of sucrose synthetase, 6·7-fold of alkaline invertase and 2·8-fold of starch phosphorylase (Table 4).

In the assay of phosphohexoisomerase, the homogenate prepared in Triton X-100 proved better than others in eliciting activity. However, incorporation of Triton X-100 led to complete inactivation of sucrose phosphate synthetase activity and lowered sucrose synthetase activity.

Lower Pseudostem

Optimal activities of sucrose phosphate synthetase, alkaline invertase, β-amylase and acid phosphatase were obtained with the homogenate containing PVP; wherein the activities were 2, 1·8, 2·7 and 1·8-fold higher respectively compared with the basal medium (Table 5). Inclusion of PVP in the grinding medium, however, led to decreased activity of sucrose synthetase.

TABLE 4. ENZYME ACTIVITIES IN BANANA ROOTSTOCK

Enzyme	Enzyme activity, units/g fresh wt.		
	Basal medium	Basal medium + 1% PVP	Basal medium + 1% Triton X-100
Sucrose synthetase	4·45	8·85	1·10
Sucrose phosphate synthetase	2·40	2·02	Nil
Acid invertase	Nil	Nil	Nil
Alkaline invertase	0·28	1·87	0·28
β-Amylase	Nil	Nil	Nil
Starch phosphorylase	1·25	3·46	1·73
Acid phosphatase	Nil	0·90	0·90
Alkaline phosphatase	Nil	Nil	Nil
Phosphohexoisomerase	16·01	23·03	27·82

The basal medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine and 0·01 M EDTA.

The activities of sucrose synthetase, starch phosphorylase and acid phosphatase were maximum in the homogenate containing Triton X-100, wherein the activities were 1·5, 1·3 and 5·4-fold higher respectively compared with the activities in the homogenate prepared in the basal medium. No alkaline phosphatase activity could, however, be demonstrated in the homogenate prepared in Triton X-100 supplemented medium.

Upper Pseudostem

The effect on enzyme activities of the upper pseudostem on incorporation of PVP or Triton X-100 in the homogenizing medium was similar to that for the lower pseudostem, with the exception of alkaline phosphatase and β-amylase. Alkaline phosphatase activity increased by 1·9-fold on incorporation of PVP in the grinding medium (Table 6).

β-Amylase activity was lost when the homogenization was carried out in the presence of PVP or Triton X-100. The activity of phosphohexoisomerase was decreased by 18% on incorporation of Triton X-100 in the basal medium.

TABLE 5. ENZYME ACTIVITIES IN BANANA LOWER PSEUDOSTEM

Enzyme	Enzyme activity, units/g fresh wt.		
	Basal medium	Basal medium + 1% PVP	Basal medium + 1% Triton X-100
Sucrose synthetase	2·90	2·20	4·75
Sucrose phosphate synthetsae	3·35	6·70	3·35
Acid invertase	Nil	Nil	Nil
Alkaline invertase	7·50	13·75	8·48
β-Amylase	0·50	1·37	1·25
Starch phosphorylase	1·32	1·32	1·73
Acid phosphatase	0·50	0·90	2·72
Alkaline phosphatase	0·93	0·93	Nil
Phosphohexoisomerase	13·91	19·24	19·24

The basal medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine and 0·01 M EDTA.

TABLE 6. ENZYME ACTIVITIES IN BANANA UPPER PSEUDOSTEM

Enzyme	Enzyme activity, units/g fresh wt.		
	Basal medium	Basal medium + 1% PVP	Basal medium + 1% Triton X-100
Sucrose synthetase	4·00	2·20	5·60
Sucrose phosphate synthetase	3·35	4·40	3·35
Acid invertase	Nil	Nil	Nil
Alkaline invertase	4·75	10·75	4·25
β-Amylase	1·03	Nil	Nil
Starch phosphorylase	1·15	0·81	1·18
Acid phosphatase	0·45	0·92	2·72
Alkaline phosphatase	0·92	1·78	Nil
Phosphohexoisomerase	18·22	17·11	15·02

The basal medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine and 0·01 M EDTA.

Fruits

β-Amylase and alkaline phosphatase activity could not be demonstrated in the homogenate prepared in the basal medium; it was essential to supplement with either PVP or Triton X-100. Maximum activities of sucrose synthetase and starch phosphorylase were obtained when PVP-supplemented medium was employed; the supplementation resulted in enhancing the activities by 3 and 15-fold respectively (Table 7).

Triton X-100 and PVP were equally effective in eliciting or increasing the activities of β-amylase and acid and alkaline phosphatase. Triton X-100 was more efficient than PVP in eliciting the optimal activities of acid and alkaline invertase and phosphohexoisomerase. The homogenate prepared in Triton X-100 supplemented medium yielded 1·5, 4·8 and 2·5-fold higher activities of acid invertase, alkaline invertase and phosphohexoisomerase compared to the homogenate in the basal medium.

DISCUSSION

The medium for extracting enzymes from plant tissues rich in phenolics requires careful formulation in order to recover maximum activity in cell-free extracts. Plant enzymes are reported to be inhibited by endogenous phenolics *per se*[5,6] and by the oxidized and polymerized phenolics.[7]

The present investigation reveals a definite relation between PVP and phenolic inhibition for at least some enzymes. Acid phosphatase activity in rootstock and leaf-sheaths, alkaline phosphatase activity in leaves and fruits, β-amylase activity in fruits and sucrose phosphate synthetase and phosphohexoisomerase in leaf-sheaths could not be demonstrated in homogenates without the supplementation of PVP in the grinding medium. Leaves which were richest in phenolics manifested increased activity of sucrose synthetase, alkaline invertase and starch phosphorylase when the basal medium was supplemented with PVP. Fruits ranked second in the phenolics content and here all the enzymes tested with the lone exception of sucrose phosphate synthetase showed increased activity on supplementation

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TABLE 7. ENZYME ACTIVITIES IN BANANA FRUITS

Enzyme	Enzyme activity, units/g fresh wt.		
	Basal medium	Basal medium + 1% PVP	Basal medium + 1% Triton X-100
Sucrose synthetase	5·70	16·80	11·20
Sucrose phosphate synthetase	5·70	2·20	5·60
Acid invertase	3·20	4·25	4·75
Alkaline invertase	1·19	5·03	5·65
β-Amylase	Nil	0·38	0·38
Starch phosphorylase	0·58	8·65	2·31
Acid phosphatase	1·38	8·29	8·30
Alkaline phosphatase	Nil	2·76	2·76
Phosphohexoisomerase	12·81	23·52	32·14

The basal medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine and 0·01 M EDTA.

of PVP. In rootstock, which contained about half the amount of phenolics present in leaves, the supplementation of the homogenizing medium with PVP increased the activities of sucrose synthetase, alkaline invertase, starch phosphorylase and phosphohexoisomerase. The activities of alkaline invertase, β-amylase and starch phosphorylase in leaf-sheaths were also increased on supplementation of PVP. Some of the enzymes of the lower and upper pseudostem (containing the lowest amount of phenolics) showed slightly higher activity on incorporation of PVP. The enhancement of many enzyme activities by PVP was likely to be due to the 'fixing' or 'sequestering' of inhibitory tannins,[6] and also by inactivation of phenolases.[8]

The efficacy of Triton X-100 depended on the particular enzyme under study and the particular tissue. Triton X-100 proved better than PVP in eliciting sucrose synthetase activity in upper and lower pseudostem, acid invertase in leaves and leaf-sheaths, alkaline invertase in leaf-sheaths, starch phosphorylase and acid phosphatase in upper and lower pseudostem, and phosphohexoisomerase in leaves, fruits and rootstock. The results with Triton X-100 may also be explained if these enzymes are present in a latent state. Triton X-100, however, produced complete inactivation of sucrose phosphate synthetase in leaf-sheaths and rootstock, of β-amylase in leaf-sheath and of alkaline phosphatase in leaves, upper and lower pseudostem. Khan *et al.*[9] observed that prolonged contact of mitochondrial enzymes with Triton X-100 in some cases led to a lowering of the degree of stimulation or even inhibition of activity.

The present investigation revealed that appropriate concentrations of PVP and Triton X-100 prevent the inactivation of some enzymes during extraction from tissues of the banana plant with cysteine solution. In view of the variability of the nature and the amount of phenolics, the location and activity of phenolases, the susceptibility of the enzyme being extracted to products of the diphenol oxidase reaction, PVP or detergents, the only definite way of arriving at the optimum composition of the dispersion medium for a given enzyme and a given tissue is by actually experimenting with all the differing media.

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EXPERIMENTAL

Plant tissues. Banana plants (*Musa paradisiaca*, cooking variety) were raised under natural conditions. A mature plant was cut at ground level when the inflorescence emerged and the first four hands of the fruit bunch (total potential of 16 hands), with about 20 fingers each were exposed. The skin of the raw banana fruits was discarded; the flesh sliced longitudinally and the central core carrying the seeds discarded. The core of the pseudostem (3·5 m long) obtained after removal of the leaf-sheaths was tender and white. The upper 1 m of the core of pseudostem comprises the upper pseudostem and the lower 1 m the lower pseudostem. The leaf-sheath used for analysis was that immediately surrounding the core of the pseudostem. The blades from 3 leaves were used in analysis after discarding the midribs; these leaves immediately preceded the inflorescence and included the atypical leaf heralding the flower. The rootstock was dug out of the ground, washed free of soil and the outer cortical region cut and discarded.

Preparation of homogenates. Each tissue was cut into small pieces, which were randomized and used fresh. Homogenates 20% (w/v) were macerated at full speed in a chilled Waring blendor for 0·5 min, off for 0·5 min and on for a further 0·5 min. Homogenization for a total of 1 min was optimal; with longer periods some of the enzymes were inactivated.

Media employed. The basal grinding medium consisted of 0·05 M Tris-HCl buffer, pH 7·0, 0·02 M freshly neutralized cysteine hydrochloride and 0·01 M EDTA. Buffering was essential because of the acidity which otherwise developed during homogenization. The extracts were filtered through muslin. Cysteine tends to keep phenolics in the reduced condition.[10,11] The use of EDTA in the homogenizing medium may be expected to inhibit the metal-mediated enzymic or non-enzymic oxidation of phenolics.[12] The concentrations of cysteine and EDTA chosen favoured maximum activity of the enzymes studied.

Polyvinylpyrrolidone supplemented medium was prepared by dissolving 1 g soluble PVP, mol. wt. 40,000, in 100 ml of the basal medium. The maximum enzyme activities were obtained at 1% PVP level; higher concentration led to inactivation of enzymes.

Triton X-100 supplemented medium was prepared by dissolving 1 ml of the detergent in 100 ml of the basal medium. Detergents solubilize enzymes,[11] and also rupture the linkage formed between phenolics and protein.[13] Maximum enzyme activities were obtained with 1% Triton X-100.

Enzyme assays. The assay mixture for sucrose phosphate synthetase was the same as for sucrose synthetase,[14] but instead of fructose, 2·0 μmoles of fructose 6-phosphate was taken. NaF (0·01 M) was added to inhibit phosphatase activity. A unit of enzyme was equivalent to the formation of 1 μmole of sucrose or sucrose 6-phosphate in 30 min at 37° and pH 7·2.

The assay for invertase was based on the method of Hatch and Glasziou,[15] with slight modification. The assay mixture consisted of 0·1 ml of 0·2 M sucrose, 0·7 ml of 0·1 M phosphate buffer, pH 7·1 or 0·1 M acetate buffer, pH 5·5, 0·2 ml enzyme preparation and water. It was incubated at 30° for 30 min. Sucrose was added to control tubes after the reaction had been stopped by the addition of 1 ml each of 5% (w/v) $ZnSO_4$ and $Ba(OH)_2$. Reducing sugars were estimated according to Nelson[16] as modified by Somogyi.[17] A unit of enzyme was equivalent to the liberation of 1 μmole reducing sugar under the assay conditions.

The assay for starch phosphorylase was based on the method of Green and Stumpf,[18] as modified by Khanna *et al.*[4] Fluoride was used to inhibit phosphatase activity. One unit of enzyme was equivalent to the liberations of 1 μmole orthophosphate in 30 min at 30° and at pH 6·0.

The assay system for acid phosphatase consisted of 1·0 ml of 0·2 M acetate buffer, pH 5·0, 0·1 ml of 0·1 M EDTA, pH 5·0, 0·5 ml of enzyme preparation and 0·3 ml H_2O. The reaction was started by the addition of 0·1 ml of 0·1 M β-glycerophosphate, pH 5·0. The incubation was carried out for 30 min at 30° and the reaction stopped by the addition of 2·0 ml of 10% TCA. Control tubes received the substrate after deproteinization. The rest of the procedure was the same as that for phosphorylase assay.

The assay system for alkaline phosphatase consisted of 1·0 ml of 0·2 M Tris-HCl buffer, pH 8·0, 0·1 ml of 0·1 M EDTA, pH 7·0, 0·2 ml of 0·2 M $MgCl_2$, 0·5 ml of enzyme preparation and 0·1 ml H_2O. The reaction was started by the addition of 0·1 ml of 0·1 M β-glycerophosphate, pH 8·0. The incubation was carried out for 30 min at 30° and the reaction stopped by the addition of 2·0 ml of 10% TCA. Control tubes received the substrate after deproteinization. One unit of phosphatase was equivalent to the splitting of 1 μmole orthophosphate in 30 min at 30°.

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The assay system for phosphohexoisomerase was based on that of Gibbs and Turner[19] with slight modification. The assay system, contained in centrifuge tubes, consisted of 0·4 ml of 0·5 M Tris-HCl buffer, pH 7·5, 0·2 ml of enzyme preparation and 0·2 ml H_2O. The reaction was started by the addition of 0·2 of 0·2 M glucose 6-phosphate, pH 7·0. The incubation was carried out for 30 min at 30°. The reaction was stopped by heating at 100° for 5 min. The control tubes received the substrate after deproteinization. Water (3 ml) was added to each centrifuge tube and after centrifugation 0·5 ml aliquots taken for the determination of fructose ester according to Roe and Papadopoulos.[20] One unit of enzyme was equivalent to the formation of 1 μmole fructose ester in 30 min at 30° and pH 7·5.

The reaction mixture for β-amylase consisted of 1·0 ml of 0·1 M acetate buffer, pH 4·5, 0·5 ml of 1% starch, 0·2 ml of enzyme and 0·3 ml H_2O and was incubated for 30 min at 30°. The enzyme reaction was stopped by the addition of 1 ml each of $ZnSO_4$ (5%, w/v) and $Ba(OH)_2$ (5%, w/v), centrifuged and aliquots taken for the determination of reducing sugar.[17] A unit of enzyme liberated reducing sugar equivalent to 1 μmole maltose under the above conditions.

Determination of total phenolics. Each tissue (leaf blades, leaf-sheaths, rootstock, lower and upper pseudostem and fruits) (20 g) were ground in a Waring blendor for 3 min with 95% EtOH (final concentration 80%). The ethanolic suspensions were quickly taken to boiling and refluxed on a steam bath for 4 hr, the extracts filtered and the residues re-extracted twice with 80% EtOH for a total period of 3 hr. The extracts from each set were pooled. In suitable aliquots of the filtrate total phenolics content was determined according to Goldstein and Swain[21] with the reagent of Folin and Denis, using tannic acid as standard.

Reproducibility of determinations. The reproducibility of the results was established by assays carried out with tissues from other banana plants.

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