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CHANGES IN ENZYME ACTIVITY OF WHITE YAM TUBERS AFTER PROLONGED STORAGE

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Key Word Index—*Dioscorea rotundata*; tuber; phosphorylase; hexokinase; glucose-6-phosphate dehydrogenase; lactate dehydrogenase; alcohol dehydrogenase.

Abstract—There is a substantial increase in the activities of phosphorylase, hexokinase, glucose-6-phosphate dehydrogenase and alcohol dehydrogenase in white yam tubers as they age. The high glucose-6-phosphate dehydrogenase activities suggest that the pentose phosphate pathway is important in yam tuber tissue.

INTRODUCTION

Yam tubers undergo considerable losses in weight during storage [1, 2] and according to Coursey [1] up to one third is attributable to loss of dry matter by increased respiration. It is known [3–8] that slices of other storage tissues such as potato [4, 6–8] carrot [3] and swedes [9] when aged artificially, for instance, by aeration in calcium sulphate or distilled water, undergo induced respiration. It has been suggested that with this type of ageing, the respiratory process becomes more efficient and faster and indeed that the respiration pathway is altered as the tissue ages [3, 4, 6]. Increases in the activities of enzymes associated with carbohydrate breakdown have been reported in potato tuber slices when aged [9–13] or wounded [14, 15] or infected with fungal pathogens [14]. Many workers [3, 5–9, 14] who have studied metabolic changes in ageing potato tubers have not indicated the ages of the tubers used (except Romberger and Norton [4]). Since it may be expected that metabolic changes occur even in the dormant intact potato tuber tissue, this is regarded as a serious omission in these studies. Thus it is generally observed that white yam tubers become sweeter in taste with age [16].

Enzyme changes might therefore be expected as the yam tubers grow older from the time of harvest. We therefore examined differences in the activities of certain enzymes of carbohydrate metabolism in physiologically old and new white yam tubers. Comparisons between white yam and other yam species of about the same age were also made. In parts of Eastern Nigeria yams are grown once a year and the early harvests begin in mid-July onwards for yams planted in November to December the previous year, whilst those planted in March to April are harvested from August to November the same year [17]. The 'old' yam tubers used in this study are those harvested around December 1975 and were used towards the end of the storage period, in October, 1976. The 'new' yam tubers are those harvested in October 1976.

Table 1. Protein concentration and enzyme specific activities of old and new white yam tubers

Enzyme	EC No.	Protein concentration mg/g fr. wt of yam tubers		Specific activity (units/mg protein)	
		old yam	new yam	old yam	new yam
Phosphorylase	2.4.1.1.	23.5	10.4*	26.5	13.3
Hexokinase	2.7.1.1.	21.0	15.3*	0.038	0.026
Glucose-6-phosphate dehydrogenase	1.1.1.49	23.0	15.4	0.30	0.11
Lactate dehydrogenase	1.1.1.27	23.0	15.4	No activity	No activity
Alcohol dehydrogenase (in the direction of alcohol oxidation)	1.1.1.1.	23.0	15.4	0.03	0.02
Alcohol dehydrogenase (in the direction of aldehyde reduction)		23.0	15.4	0.17	0.14

* Differences in protein concentration are probably attributable to solvent effects since protein determinations were made with different buffer systems (see Experimental).

Table 2. Changes in enzyme activities in old and new white yam tubers

Enzyme	Enzyme activity μmol product/min/g fr. wt yam tubers				Percentage change in enzyme activity old yam/new yam
			Mixture (1:1 Old and new yam tubers)		
	old yam	new yam	obtained	expected	
Phosphorylase	39.5	8.64	16.4	24.2	458
Hexokinase	0.63	0.31	0.47	0.47	203
Glucose-6-phosphate dehydrogenase	5.55	1.35	4.10	3.45	411
Lactate dehydrogenase	No activity	No activity	No activity	—	—
Alcohol dehydrogenase (in the direction of alcohol oxidation)	0.48	0.20	0.31	0.34	240
Alcohol dehydrogenase (in the direction of acetaldehyde reduction)	3.06	1.68	2.22	2.37	182
Activity ratio of alcohol oxidation/ acetaldehyde reduction	0.16	0.12	0.14	0.14	—

RESULTS AND DISCUSSION

Table 1 shows the protein concentration and enzyme sp. act. of old and new white yam tubers, while Table 2 shows the relative increases in activity of the various enzymes of white yam tuber with age. Enzyme activity has been expressed per g fr. wt of yam tubers in order to enable comparison to be made with results obtained by other workers. Table 1 shows that the old yam tubers contain more protein per g and also possess higher sp. act. for every enzyme studied than the new white yam tubers. It can be seen from Table 2 that all enzymes studied showed marked changes in activity as the white yam tuber ages. In every case enzyme activity was also determined for mixtures of equal wts of old and new white yam tubers and the values obtained compared with the values expected by a computation from the separate activities. These results are shown in Table 2 where it can be seen, that there is reasonable agreement between the results obtained and those expected. This indicates that the observed differences are real and are not due to enzyme inactivation [18] nor due to incomplete extraction [19].

The increases in activities of both glucose-6-phosphate (G-6-P) dehydrogenase and alcohol dehydrogenase are comparable with values reported by Sacher *et al.* [9], who subjected potato and swede discs to artificial ageing. The increases in hexokinase and G-6-P dehydrogenase activities obtained here for physiologically aged white yam tuber tissues are of the order of those reported by Ricardo and Ap Rees [19] with discs of carrot tissue aged artificially for 24 hr.

The results reported here for aged white yam tubers show a 2-fold increase in hexokinase and a 4-fold increase in G-6-P dehydrogenase activity with ageing. The relatively high activity of G-6-P dehydrogenase tends to suggest that the pentose phosphate pathway is important in yam tubers and that the contribution of this pathway to carbohydrate metabolism increases with age. Phosphorylase activity was over 4-fold more in old than in new white yam tuber tissue. However, in previous studies carried out in June–July 1975 phosphorylase was over 40 fold higher in old than in new white yam tubers whilst hexokinase was 11 fold higher. All other enzymes studied showed similarly more marked increases in activities of old over new white yams as measured in June–July 1975 when compared with the values obtained from experiments conducted in October 1976. It is commonly observed that in Eastern Nigeria yams reach their sprouting peak and presumably 'metabolic activity peak' in May–June–July and it is significant that those preliminary studies suggest that the levels of many enzymes are higher in July than in October. By October any surviving old yams have become metabolically 'quiescent' and this seems to be in agreement with the observed reduction in activities of all enzymes. Studies in this laboratory are now being carried out to follow the changes in enzyme levels periodically during yam storage.

Phosphorylase may be expected to play a key role in providing glucose phosphates to the various pathways of carbohydrate degradation. It has for instance been suggested that formation of G-6-P through the phosphorylase/phosphoglucomutase reactions in some tissues is more important than the production by hexokinase action [20]. Hexokinase may serve to convert any free glucose produced from yam starch to G-6-P. G-1-P produced by the action of phosphorylase could also be converted to free glucose by phosphatase action, thus increasing the free sugar concentration in white yam tuber tissue. This probably contributes to the sweet taste observed in yam tubers after prolonged storage [16].

The physiological role of alcohol dehydrogenase is uncertain but it is thought to function in the reduction of acetaldehyde formed under anaerobic conditions. In potato tubers it is thought [21] that little acetaldehyde arises in this way since potato tubers contain little or no pyruvic decarboxylase [22]. Thus it has been shown that under anaerobic conditions potato tubers form lactic acid and not ethanol [23]. Similar studies have not been carried out with yam tubers, but whatever the role of alcohol dehydrogenase is in yam tubers, old yam tubers show higher levels of the enzyme than the new yams. However, the activity ratios of alcohol

Table 3. Enzyme activities in old *D. rotundata*, *D. alata* and *D. cayenensis* tubers

Enzyme	Enzyme activity (μmol product/min/g fr. wt of yam tubers)		
	D. rotundata	*D. alata*	*D. cayenensis*
Hexokinase	0.63	0.24	0.06
Glucose-6-phosphate dehydrogenase	5.55	0.72	1.78
Alcohol dehydrogenase (in the direction of alcohol oxidation)	0.48	0.03	0.22
Alcohol dehydrogenase (in the direction of aldehyde reduction)	3.06	0.02	1.16

oxidation/acetaldehyde reduction of 0.16, 0.12 and 0.14 for old, new, and old plus new yam tuber mixture respectively seems to indicate that there is no change in enzyme form, since this ratio varies with different alcohol dehydrogenases [24]. It is significant that in every case these values are about half of the corresponding values obtained in the June–July experiments of 1975. Lactic dehydrogenase activity was assayed in our work using the method of Davies and Davies [25] in the direction of pyruvate reduction, but no activity was detected either with the old or new white yam tubers. Agughasi [26] has demonstrated some lactic dehydrogenase activity using a modified method and *D. cayenensis* as enzyme source. A comparison of the activities of hexokinase, G-6-P dehydrogenase and alcohol dehydrogenase in white yam (*D. rotundata*), water yam (*D. alata*) and yellow yam (*D. cayenensis*) is shown in Table 3. It can be seen that in every case the level of activity of all 3 enzymes is significantly higher in *D. rotundata* than that in both *D. alata* and *D. cayenensis*. The significance of this observation in the storage life of the different yams may be worthy of further investigation.

EXPERIMENTAL

Material. The 'old' yams used in this study were obtained from the Department of Crop Science, University of Nigeria, Nsukka. They were harvested at the end of the previous growing season and stored in the conventional manner on stacks in a covered barn. The 'new' yams were harvested in the current growing season. 'Old' and 'new' yams were kept in an air-conditioned room at 20° for at least 48 h before use.

Preparation of yam discs. 4–6 cm thick discs were cut from the middle of the yam tuber. Using a No. 7 corkborer (internal diameter 1 cm) cylinders of tissue were obtained from these discs and from them smaller discs were cut, washed in cold H_2O and weighed in ice-cold $Na_2S_2O_3$ soln (7 g/l.) contained in a beaker. Any defective areas, found especially on the old yam tubers, were avoided.

Preparation of crude enzymes. For extraction of phosphorylase, 5 g of yam discs washed in cold H_2O was homogenized in 50 ml extraction mixture consisting of 35 mM glycerol-2-phosphate, 20 mM NaF, mM EDTA and 30 mM mercaptoethanol, pH 6.2 as described in ref [27]. The resulting homogenate was squeezed through cheese-cloth and the extract centrifuged at 24000 *g*. The resulting supernatant was stored at 4° and used as the crude enzyme. For extraction of hexokinase 5 g samples of yam tuber discs were homogenised in 50 ml of an extraction mixture consisting of 50 mM triethanolamine, mM EDTA, 2 mM $MgCl_2$ and 30 mM mercaptoethanol, pH 7.5. The homogenate was squeezed through cheese-cloth and the extract centrifuged at 24000 *g* for 10 min. The resulting supernatant was used for the hexokinase assay. For G-6-P dehydrogenase, lactate dehydrogenase and alcohol dehydrogenase extraction, 5 g discs were homogenised in 50 ml borate buffer (50 mM, pH 8.4). After squeezing the homogenate through cheese-cloth the extract was centrifuged at 24000 *g* for 10 min and the supernatant used immediately for assay of G-6-P, lactate and alcohol dehydrogenase activities. In every case the enzyme prepn was performed in duplicate. Enzyme extraction for the old plus new yam mixture was done on 5 g yam discs consisting of 2.5 g old and 2.5 g new yam. The vol. of the supernatant recovered was recorded in every case.

Protein determination. Protein was determined using the method of ref [28].

Enzyme assay. Phosphorylase was assayed using a modification of the method described in ref [27] in the direction of starch synthesis. 0.2 ml of 50 mM G-1-P, 1 ml of 0.5 mM AMP, 0.5 ml of satd starch soln were pipetted into each of 3 test tubes. H_2O was added to make each up to 2 ml and the mixture incubated at 35° for 15 min before 1 ml of enzyme extract was added. The tests were done in triplicate in every case, G-1-P and AMP being left out from the control tubes. After 15 min of further incubation with enzyme the reactions were stopped by the addition of 1 ml of 6% $HClO_4$. The reaction mixtures were then centrifuged at 5000 *g* for 5 min and the clear supernatant collected. 0.5 ml samples of the resulting supernatants were then used for determination of P_i by the method of ref [29].

Hexokinase was assayed as described in ref [27] except that 0.5 ml of the extract was used. G-6-P dehydrogenase was determined according to a method described in ref [30], with slight modifications. This method involved, first measuring 6-phosphogluconate (6-PG) dehydrogenase activity at pH 7.6 using Tris buffer (M, pH 7.6) 0.3 ml; $MgCl_2$ (M), 0.3 ml; H_2O 1.6 ml; NADP (2 mM), 0.3 ml; 6-PGA (6 mM), 0.3 ml; and enzyme 0.2 ml, in a total vol. of 3 ml.

6-PG and G-6-P dehydrogenase activities were measured together by using 0.3 ml 6-PGA (6 mM), 0.3 ml G-6-P (6 mM), 1.3 ml H_2O and the same amounts of every other reagent as for 6-PG dehydrogenase activity assay in a total vol. of 3 ml. Increase in *A* at 340 nm was measured over 5 min and G-6-P dehydrogenase activity taken as the difference between the two activities obtained as above. Under the above conditions the rate of NADP reduction was linear, whereas the method of ref [30] did not give a linear trace for more than 2 min.

Lactate dehydrogenase was assayed in the direction of pyruvate reduction using the method described in ref [25]. Alcohol dehydrogenase was determined by a modification of the methods described in ref [31] and [32] using NAD^+ (0.015 M), 0.1 ml; Tris buffer (0.1 M pH 8.8), 0.5 ml; EtOH (5 M), 0.1 ml; H_2O 1.8 ml and enzyme 0.5 ml. For the reverse reaction the mixture consisted of NADH (3×10^{-3} M), 0.1 ml;

Tris buffer (0.1 M pH 7.4), 0.5 ml; MeCHO (3×10^{-2} M), 0.1 ml; H_2O 1.8 ml and enzyme 0.5 ml. In each case the determinations were made at least 3 times and the results shown are the means of these determinations.

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