Phytochemistry, 1971, Vol. 10, pp. 1007 to 1009. Pergamon Press. Printed in England.

SHORT COMMUNICATION

BIOCHEMICAL STUDIES ON CHILLING INJURY IN MANGOES

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(*Received* 4 *April* 1970, *in revised form* 24 *July* 1970)

Abstract—The development of chilling injury in mango peel, as in the pulp is marked by a significant decrease in the soluble sugar content (mainly sucrose), no significant change in total hexose content and less starch breakdown; in addition, invertase activity increases whereas that of amylase decreases. Mango invertase shows two temperature optima, one at 0° and the other at 37°.

INTRODUCTION

MANY fruits and vegetables are injured physiologically at storage temperatures considerably above the freezing point of the tissues[1–4] and this injury is termed 'chilling injury'. The development of chilling injury restricts the storage of fruits at low temperatures; therefore, in recent years the reactions that are involved in the mechanisms of this abnormality have been the subject of considerable interest.[2–7] Though the symptoms of chilling injury have been described adequately in many tissues there have been few metabolic and physiological studies of this phenomenon. Earlier, we studied[8] the changes in chemical constituents and the glycohydrolytic enzymes, and the effect of the accumulated minerals on enzyme activities during the development of chilling injury in mangoes. Further investigations have been carried out to find out the effect of temperature on invertase in peels of chill injured and healthy tissues.

RESULTS AND DISCUSSION

We have observed that in the peels there is a marked change in some of the cellular constituents during chilling injury. The results (Table 1) show that during chilling injury there is more degradation of sucrose but less degradation of starch as compared to the healthy peels. These results are substantiated by the increase in the activity of the enzyme invertase and the decrease in that of amylase during chilling injury (Table 1). Moreover no marked change was observed in the protein content and free hexose of the affected and non-affected tissue. Such a pattern of changes in peels was observed at all the stages of ripeness.

[1] F. KIDD and C. WEST, *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Botan. Dept.* (1932) **57**, 82 (1933) and 117 (1934).
[2] W. W. JONES, *Plant Physiol.* **17**, 481 (1942).
[3] O. A. LORENZ, *Proc. Am. Soc. Hort. Sci.* **57**, 288 (1951).
[4] E. V. MILLER, *Plant Physiol* **26**, 66 (1951).
[5] J. B. BIALE, *Proc. Soc. Hort. Sci.* **39**, 137 (1941).
[6] J. B. BIALE, R. E. YOUNG and A. OLMSTEAD, *Plant Physiol.* **29**, 168 (1954).
[7] M. LIEBERMAN, C. C. CRAFT, W. V. ANDIA and M. S. WILCOX, *Plant Physiol.* **33**, 307 (1958).
[8] A. K. MATTOO and V. V. MODI, *Proc. Int. Conf. Trop. and Subtrop. Fruit*, T.P.I. London Sept. (1969) III (1970).

TABLE 1. CHANGES IN THE CONTENTS OF FREE HEXOSES, TOTAL SOLUBLE SUGARS, STARCH, AMYLASE AND INVERTASE IN CHILL INJURED AND HEALTHY PEELS OF MANGOES*

Type of the peel tissue	Free hexoses (g%)	Total soluble sugar (g%)	Sucrose (g%)	Starch (g%)	Amylase (units †/mg protein)	Invertase (units†/mg protein)
Healthy	0·55	0·98	0·43	0·343	0·062	0·014
Chill-injured	0·58	0·70	0·12	0·536	0·024	0·024

* Partly ripe (climacteric) mangoes were taken for analyses. Results reported are an average of 10 determinations.

† One unit of amylase activity is that amount which liberates one mg of reducing groups calculated as maltose per 30 min at 37°. One unit of invertase activity is that amount which liberates 1 μmole of reducing hexose/hr at 37°.

These data on peels are comparable to those obtained in fruit pulp.[8] The symptoms of chilling injury appeared earlier in the peel and then in the pulp indicating that the above mentioned biochemical changes start in the peel and then follow on in the pulp. Our preliminary studies have indicated a similar series of biochemical changes in bananas during chilling injury.

Earlier[8] we demonstrated the accumulation of calcium, potassium and sodium in chill injured tissue, and have shown that *in vitro* amylase activity is inhibited by the presence of K^+ and Ca^{2+} whereas the invertase activity is stimulated considerably in their presence. An interesting observation was made while studying the effect of temperature on invertase activity that the fruit invertase shows two temperature optima; one at 0° and the other at 37° (Fig. 1). This may be one of the causes of the development of chilling injury. Further investigations are in progress.

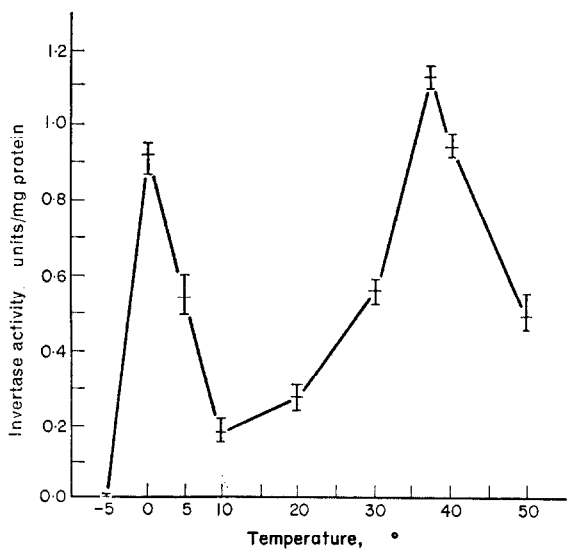

FIG. 1. EFFECT OF INCUBATION TEMPERATURE ON THE ACTIVITY OF MANGO INVERTASE. The enzyme system, as mentioned in the text, was incubated at the indicated temperatures (± 1°) for 30 min. One unit of invertase activity is that amount of enzyme which liberates one micromole of reducing hexose per hr under the experimental condition. The bars indicate variations in the data of experiments conducted.

EXPERIMENTAL

Alfanso mangoes (*Mangifera indica*), used for this study, were obtained from the Bulsar district of Gujarat State (India). The matured unripe mangoes were picked from the trees and were transported to the laboratory within 24 hr. Immediately they were washed and cleaned, and kept for ripening at 25°. The various stages of the fruit during ripening were marked by the color development and appearance: (a) unripe: green peel, firm when touched and white pulp; (b) partly ripe: green to yellow peel, slightly soft and faint yellow pulp; (c) ripe: golden yellow peel, soft when touched and yellow to golden yellow pulp. The fruits at different stages of ripeness were exposed to low temperature in a cold room maintained at 2°–5°. Chilling injury was marked by the appearance of skin blemishes and darkening of the tissue, failure of normal ripening, and lack of flavour after removal from the cold storage. The chill injured and healthy parts of the same fruit were cut and analyzed within 12 hr; the results reported are an average of triplicate determinations of ten different fruit samples, each from three different stages. Analyses of both the peel and the pulp were carried out. Methods employed for (a) estimation of sugars and starch, (b) preparation of cell free extracts and (c) enzyme assays were essentially the same as reported earlier.[8] Protein in the TCA precipitates of cell free extracts was determined by the method of Lowry *et al.*[9]

The effect of incubation temperatures on the invertase activity was determined under optimal conditions. Standardized enzyme system containing (in 2 ml), 100 μmoles sodium acetate buffer (pH 5·0), 25 μmoles sucrose and an appropriate concentration of ripe mango enzyme extract (50–600 μg protein), was incubated at $-$ 5°, 0°, 5°, 10°, 20°, 30°, 37°, 40° and 50° ($\pm$ 1°) for 30 min. Control tubes (a) without substrate, (b) without enzyme, (c) with boiled enzyme and in which (d) enzyme reaction was stopped at 0 min, were run simultaneously. Enzyme reaction was terminated by the method of Pressey[10] and the hexose liberated was estimated quantitatively by the arsenomolybdate method.[11] Results reported in Fig. 1 are an average of 30 determinations; the enzyme activity was linear with respect to time (up to 2 hr) and enzyme protein (100–600 μg).

Acknowledgement—This investigation was supported by a P1-480/Fg-In-276 grant from the Agricultural Research Service of the U.S. Dept. of Agriculture.

[9] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193,** 265 (1951).
[10] R. Pressey, *Arch. Biochem. Biophys.* **133,** 667 (1966).
[11] G. Ashwell, *Methods in Enzymology* (edited by S. P. Colowick and N. O. Kaplan), Vol. III, p. 73, Academic Press, New York (1955).