Invertase from Date Fruits

Soluble and insoluble invertase had been isolated from date fruits (Zehdi variety) after epicarp removal. The optimum temperature for both enzymes was 45 °C. The optimum pH for soluble invertase was 3.6–4.8 whereas the optimum pH for insoluble invertase was 3.6–4.2. Both enzymes had high affinity for their substrate, sucrose, with K_m values of 3.12×10^{-3} mM and 4.35×10^{-3} mM for soluble and insoluble invertases, respectively. The molecular weight of the soluble invertase was retained on the M_r 300 000 molecular filters. The specific activity of soluble invertase was 40.2 μmol (mg of protein)$^{-1}$ min^{-1} while the specific activity of insoluble invertase was 1.1 μmol (mg of protein)$^{-1}$ min^{-1}. Sodium dodecyl sulfate inhibited both enzymes.

Invertase D-fructofuranoside fructohydrolase) exists in plants in soluble (Hatch et al., 1963; Jaynes and Nelson, 1971; Pressey, 1966) and insoluble (Chin and Weston, 1973) forms. Plant invertases were found to be pH dependent. Acid invertases as well as neutral invertases were found in sugar cane (Hatch et al., 1963), pea (Maclachlan et al., 1970), and carrot (Ricardo and Rees, 1970). Invertases play a major role in the metabolism of sucrose in plant tissue (Hatch et al., 1963; Bacon et al., 1965) and cause commercial losses for the sugar industry (Alexander, 1972).

Iraq produces about 300 000 tons of Zehdi date fruits annually (Ministry of Industry, 1973). The mature date fruits contain sucrose as well as glucose and fructose, which account for 80% of its dry weight (Al-Dawody et al., 1967). In early stages of maturation Zehdi date fruits contain a high concentration of sucrose content that decreases at the time of ripening (Benjamin et al., 1975). This study deals with characterization and inhibition of invertases from Zehdi date fruits in an attempt to prevent inversion of sucrose to glucose and fructose. The reason for preventing the inversion of sucrose to glucose and fructose is to try to produce sucrose commercially from date fruits in a crystalline form; on the other hand it would be extremely difficult to crystallize inverted sugar on a technical commercial basis. One can produce inverted sugars from date fruit in a liquid form but it creates immense marketing problems.

EXPERIMENTAL SECTION

Chemicals were from the following companies: 3,5-dinitrosalicylic acid from BDH; bovine serum alubumin (BSA) from Serva; sucrose from Calbiochem; mature date fruits (*Phoenix dactylifera* L.), Zehdi variety, from Zafarania Orchid, Baghdad.

Epicarps from date fruits were removed and discarded. The date fruits were pitted. A 10-g sample was homogenized in an MSE high-speed homogenizer in 50 mL of cold 0.05 M borate buffer, pH 8.5, containing 1 M NaCl for 5 min. The homogenate was filtered through a No. 4 mesh (3–15-μm pore size) sintered glass funnel with a vacuum suction. The precipitate retained by the funnel was homogenized again in 20 mL of the same buffer and refiltered. The filterate was collected and centrifuged in a Model J21-C Beckman centrifuge at a speed of 20 000 rpm for 30 min at 2 °C. The supernatant was used as a source for crude soluble invertase. The pellet was resuspended in 20 mL of 0.05 M acetate buffer, pH 4.2, and centrifuged again. The supernatant was discarded and the pellet was used as a source of insoluble enzyme. A portion of the 0.2-g pellet was suspended in 20 mL of 0.05 M acetate buffer, pH 4.2. This suspension was used as a source of insoluble invertase. Samples of 10 mL of crude soluble invertase supernatant were applied to Amicon MMC cell containing the following Diaflo filters: UM 10, UM 20, PM 30, XM 50, XM 100A, and XM 300 that had 10 000, 20 000, 30 000, 50 000, 100 000 and 300 000 molecular weight retentivities, respectively. Extracts retained on the filters were washed extensively with 200 mL of cold 0.05 M acetate buffer, pH 4.2, or until all proteins disappear from the filtrate. The extract retained in the MMC Amicon cell by the XM 300 membrane filter contained all soluble invertase activity and was used as a source for the soluble enzyme.

Enzyme Assays. Invertase activities were determined in a 2-mL total volume mixture containing 1 mM sucrose, 20 mM acetate buffer, pH 4.2, 0.05 mL of soluble or insoluble enzyme preparation, and distilled water. The mixtures were incubated at 37 °C in test tubes. At zero time and 10 min of incubation aliquots of 0.2-mL assay mixtures were withdrawn and pipetted into test tubes. A volume of 0.5 mL of 3,5-dinitrosalicylic acid was added to each test tube. The test tubes were placed in a boiling-water bath for 10 min. The color developed was measured at 540 nm with a Karl Zeiss Model PM 4 spectrophotometer. Amounts of reducing sugars were determined according to Bernfeld (1955). Protein contents in assay mixtures were determined according to Lowry et al. (1951) using BSA as a protein standard. Specific activity is defined as micromoles of reducing sugars produced per milligram of protein per minute.

Effect of Inhibitor. Enzyme extracts where incubated with different concentrations of $NaDodSO_4$ (0.1–0.9 μmol) and 10 μmol of sucrose. The reaction was started by the addition of the substrate (sucrose). The kinetic of $NaDodSO_4$ inhibition to both enzymes was studied by using the same concentrations of sucrose (1–10 μmol); however, 0.0, 0.08, and 0.1 μmol of $NaDodSO_4$ were used for soluble invertase while 0.0, 0.2, and 0.3 μmol of $NaDodSO_4$ were used for insoluble invertase.

RESULTS

The enzymes were obtained according to the procedure outlined under Experimental Section. The soluble invertase was retained on XM 3000 Diaflo membrane. The specific activity of the crude enzyme was 24 μmol (mg of protein)$^{-1}$ min^{-1}. While the specific activity of the M_r 300 000 fraction from the Diaflo membrane was 40.2 μmol (mg of protein)$^{-1}$ min^{-1}. The specific activity of the insoluble enzyme was 1.0 μmol (mg of protein)$^{-1}$ min^{-1}.

The effect of pH was examined on the activity of soluble and insoluble invertases by using three different buffers. Glycine–HCl, citrate, and phosphate buffers were used to cover the pH range 2.2–8.0 with overlapping points. No specific buffer effect could be observed. The pH optimums for soluble and insoluble invertases were 3.6–4.8 and 3.6–4.2, respectively (Figure 1).

Effect of Temperature. Temperature sensitivity of soluble and insoluble invertases was determined at 20, 37, 45, 50, and 60 °C. The optimum temperature for both enzymes was 45 °C. However, soluble invertase had no

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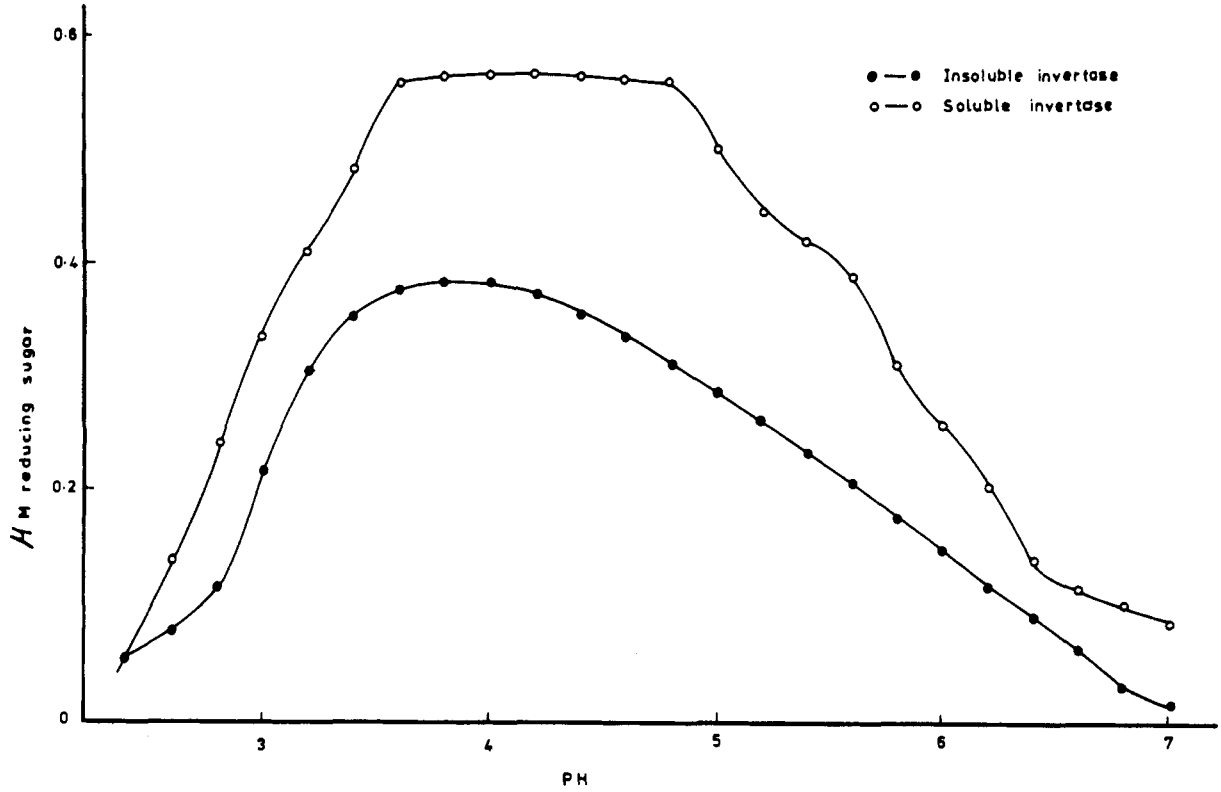

Figure 1. Effect of pH on soluble and insoluble invertases from Zehdi date fruits.

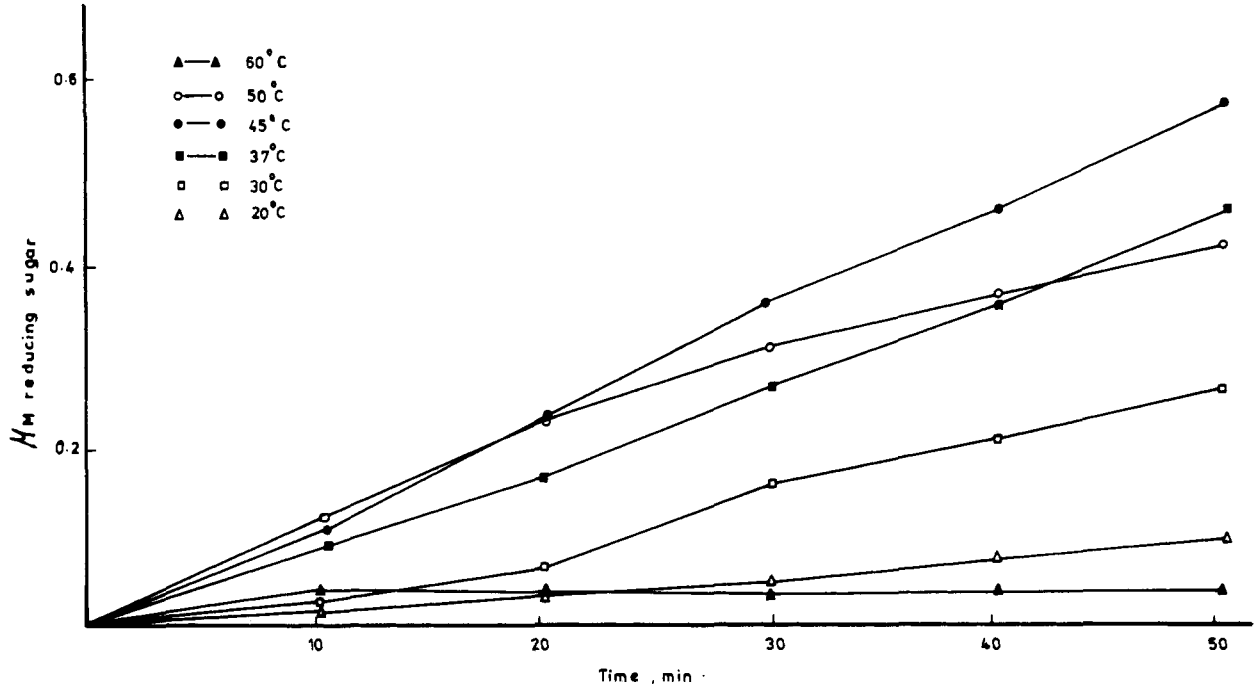

Figure 2. Effect of temperature on soluble invertase activity from Zehdi date fruits.

noticable activity at 60 °C (Figure 2), whereas the insoluble invertase activity at 60 °C was more than at 20 °C (Figure 3).

Kinetic Constants. The kinetic constants for both enzymes were determined by using different sucrose concentrations (4 nmol–1000 μmol). The K_m values were 3.12 × 10^{-3} mM and 4.35 × 10^{-3} mM for soluble and insoluble invertases, respectively, thus indicating high affinities for their substrate sucrose (Figure 4).

Insoluble invertase showed more resistance toward inhibition by $NaDodSO_4$ than soluble invertase (Figure 5). Inhibition of soluble invertase by $NaDodSO_4$ was noncompetitive (Figure 6), whereas soluble invertase was competitively inhibited (Figure 7).

DISCUSSION

As in many plant tissues Zehdi date fruit contains two forms of invertases: soluble and insoluble enzymes. The two enzymes are slightly different as can be judged from their temperature, pH, and inhibition by $NaDodSO_4$. Insoluble invertase was more active at 60 °C than soluble invertase. It is interesting to know that the optimum temperature for both enzymes was 45 °C, which is almost the same temperature occurring naturally in Iraq at the

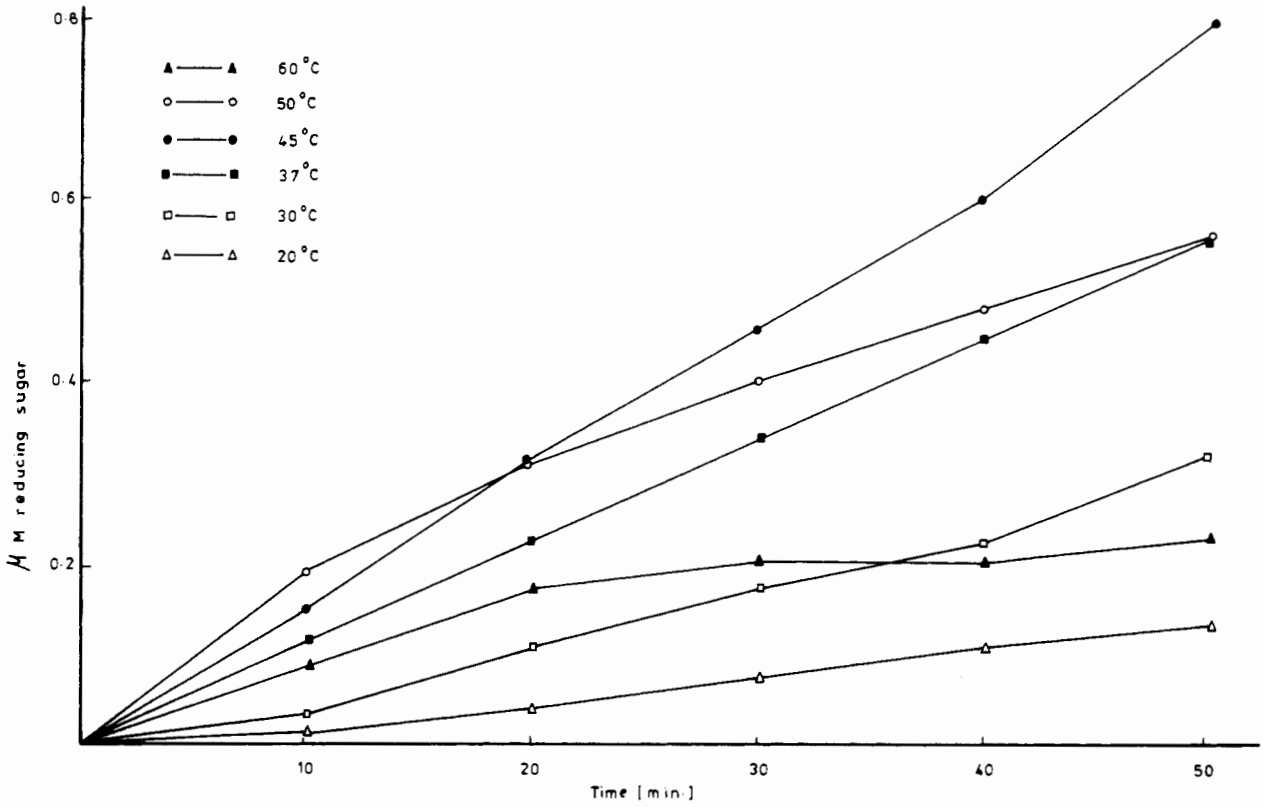

Figure 3. Effect of temperature on insoluble invertase activity from Zehdi date fruits at pH 4.2.

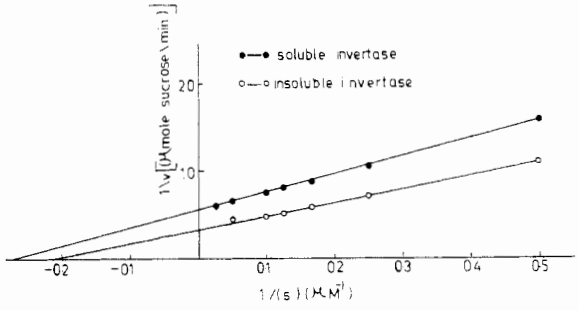

Figure 4. Lineweaver–Burk plot of enzyme activity of the soluble and insoluble invertase activities at different concentrations of $NaDodSO_4$.

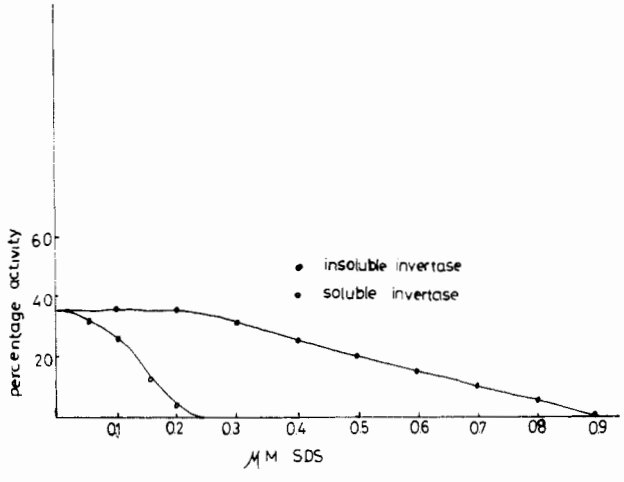

Figure 5. Percent response of soluble and insoluble invertase activities at different concentrations of $NaDodSO_4$ (SDS in the figure).

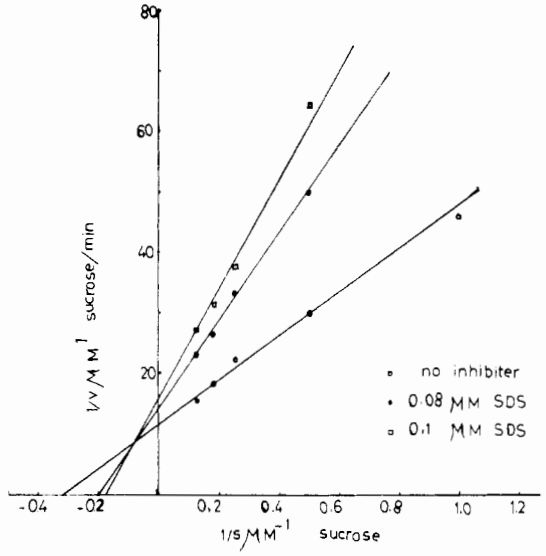

Figure 6. Double-reciprocal plots of velocity against sucrose concentrations in the presence of $NaDodSO_4$ (SDS in the figure) (soluble invertase).

time of ripening of the date. This may indicate the physiological role of these enzymes in the conversion of sucrose to glucose and fructose. This is similar to that reported by Manning and Maw (1975) for tomatoes where invertases contribute much to the inversion of sucrose.

We were not able to detect any appreciable invertase activities in the neutral range of pH. It was reported that tomato plants contain only acid invertase (Manning and Maw, 1975). However, other plants contain both acid and neutral invertases (Skukla et al., 1973).

Sodium dodecyl sulfate inhibited both soluble and insoluble invertases. It is known that $NaDodSO_4$ binds to proteins and causes then to dissociate to their subunits (Swindlehurst et al., 1977; Lunney and Sachs, 1978). Small concentrations of $NaDodSO_4$ inhibited beef heart and chicken heart dehydrogenase (Sabato and Kaplan, 1964),

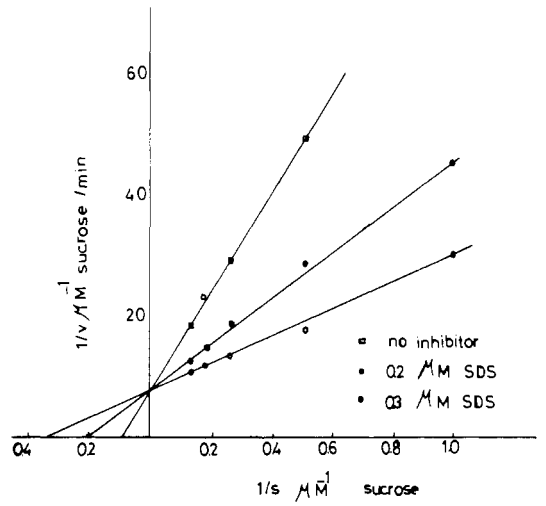

Figure 7. Double-reciprocal plots of velocity against sucrose concentrations in the presence of $NaDodSO_4$ (SDS in the figure) (insoluble invertase).

tryptophan oxygenase (Koike et al., 1969), and asperate transcarbamylase (Colman and Markus, 1972). However, some enzymes were resistant to $NaDodSO_4$ and showed no loss of activity even after 4 h of treatment with high concentrations of (Nelson, 1971).

Webb (1963) proposed an uncompetitive mechanism for the inhibition of enzymic activities by $NaDodSO_4$. Others aslo reported uncompetitive inhibition of acid and neutral invertases from sugar cane (Rosario and Santisopasri, 1977) and bovin liver glutamate dehydrogenase (Rogers and Yusko, 1972) by $NaDodSO_4$. Our data indicate $NaDodSO_4$ acted as a competitive inhibitor for the soluble enzyme and a noncompetitive inhibitor for the insoluble enzyme. In spite of our extensive review of literature we were unable to locate similar competitive inhibition by $NaDodSO_4$ to other enzymes.

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Composition of Lipids in *Dioscorea* Tubers

Lipids extracted from *Dioscorea* tubers were analyzed, and the fatty acid composition of the various lipid types was determined. Total lipid content of the four different species varied from 0.4% to 1.5%, but the percentage composition of the lipid classes was nearly the same. The quantitative composition of these lipids was in general very similar to that in many other nonphotosynthetic plant storage tissues.

The bulky storage tissue of *Dioscorea* species, commonly called yams, is important in human nutrition throughout West Africa. Environmental conditions that influence the yield and storage of this crop have been studied extensively (Coursey, 1967; Onwueme, 1978). However, the gross chemical composition of these tubers has received little attention (Oyenuga, 1968; Kay, 1971). In particular, these reports merely stated the amount of crude fat in the tuber.

With a lipid content of less than 2% of dry weight, the contribution of these tubers to total dietary fat intake may be considered negligible. However, polar lipids are essential part of the tuber tissue membranes where they form a lipoprotein complex responsible for the transport of material and permeability processes involving diffusion and active transport against a prevailing electrochemical gradient (Hitchcock and Nichols, 1971). Tubers with a higher lipid content have recently been shown to be less susceptible to damage following bruising and less susceptible to discoloration (Mondy and Koch, 1978; Klein et al., 1981). Furthermore, fat composition and changes are now included among possible quality factors in food prepared from these tubers (Grosch, 1972). This communication reports on the composition of polar lipid classes in tubers of four *Dioscorea* species. The information will provide

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