Date Invertase: Properties and Activity Associated with Maturation and Quality

Shin Hasegawa and Dora C. Smolensky

Invertase activity was found in both soluble and insoluble fractions of mature dates. Immature dates possessed only the insoluble invertase. The soluble invertase, which was virtually absent at the green stage, began to develop as maturity progressed. The dramatic increase in the soluble invertase occurred during the period that the fruit matured from the green to early red stages. The insoluble invertase, on the other hand, decreased approximately 50% during this period, but remained fairly constant after the early red stage. Among the four grades of dates used in this study, soft and good quality dates had activities higher than those of tougher dates. Both soluble and insoluble invertase attacked sucrose, raffinose, and melezitose in a similar manner. Treatments with various concentrations of Carbowax 4000 and Tween 80 had practically no effect on solubilization of the insoluble invertase.

Texture is perhaps the prime factor which determines the quality of dates. During the course of our investigation on enzymes related to changes in texture during fruit ripening, we found that dates possess a significant amount of polygalacturonase activity during late stages of development, and that polygalacturonase activity and softening of date tissues are closely related to each other (Hasegawa *et al.*, 1969). We suggested that polygalacturonase is involved in controlling the texture of dates.

Dates contain very high concentrations of sugars—mainly sucrose, glucose, and fructose. Soft, high grades usually have a ratio of reducing to total sugar contents higher than those of tough, low quality (Cook and Furr, 1953; Maier and Metzler, 1961; Sinclair *et al.*, 1942). Maier and Metzler (1961) observed the importance of sucrose hydrolysis on the textural quality of dates. The degree of this inversion was a function of invertase activity, moisture content, and the temperature of incubation.

In this paper we report the results of our investigation on how invertase (β-D-fructofuranoside fructohydrolase, E.C.-3.2.1.26) activity changes during fruit development, and how this activity is related to the quality of dates. Some properties of invertase are also reported.

MATERIALS AND METHODS

Dates (*Phoenix dactylifera* L., var. Deglet Noor) used in this study were the same samples used previously for polygalacturonase analysis. Description of the dates, including their appearance and some chemical and physical characteristics, was given in a previous publication (Hasegawa *et al.*, 1969). Glucose oxidase and peroxidase were purchased from Worthington Biochemical Corp., Freehold, N. J.

Invertase was extracted from the fruit by the following procedure. Six to ten dates were pitted, ground in a food chopper, and mixed. A 10-g sample was weighed and macerated with 100 ml of a 4.0% NaCl solution in an Omnimixer for 2 min. One gram of insoluble polyvinylpyrrolidone was used also to prevent the formation of protein-tannin complexes. The mixture was centrifuged at 20,000 G for 15 min The residue obtained was resuspended in 100 ml of the same solution and the extraction was repeated. A 10-ml portion of the combined extracts was dialyzed against running distilled water at 2° C until sugars were removed completely. This dialyzate was used as soluble invertase. On the other hand, the residue obtained above was washed with distilled water sufficient to remove all sugars remaining in the tissues, and this sugar-free residue was used as insoluble invertase. All the preparative procedures were carried out below 5° C.

Routinely, enzymic reaction mixtures (4 ml) consisted of 0.1 M acetate buffer, pH 4.5, 2.5×10^{-3} M sucrose and approximately 5×10^{-3} unit of invertase. The mixture was incubated at 30° C. One ml of each sample was taken at 10-min intervals, and the increase in glucose formation was followed enzymatically by the modified procedure of Messer and Dahlqvist (1966). To 1 ml of the reaction mixture was added 1 ml of the glucose oxidase-peroxidase reagent consisting of 0.2 M phosphate buffer, pH 7.0, 1 unit of glucose oxidase, 3 units of peroxidase, and 100 ppm of *O*-dianisidine. The mixture was then incubated at 30° C for 30 min, at the end of which time the reaction was stopped by addition of 3 ml of 50% sulfuric acid and the absorbancy at 530 mμ was measured by a Cary 14 spectrophotometer. Concentrations of 0.01 to 0.1 μmole of glucose were measured under the above conditions. One unit of invertase was defined as the amount of enzyme which catalyzes the production of 1 μmole of glucose per min under the above conditions.

RESULTS

Extraction of Invertase. Extractions were tried with various concentrations of NaCl solution. The use of NaCl in the extracting solution resulted in extracts with high enzyme activity in the soluble fraction, as compared to extracts without NaCl (Figure 1). The activity in the soluble fraction increased as the salt concentration increased, and reached its maximal level approximately at a concentration of 3.0%.

The results of investigation on solubilization of the insoluble invertase with various concentrations of Carbowax 4000 and Tween 80 are shown in Table I. These compounds, which are known to break tannin-protein complexes, had practically no effect on solubilization of the insoluble invertase.

Properties. The results of measurement of invertase activity at various hydrogen-ion concentrations show that invertase had an optimal pH at 4.5 and no alkaline invertase

Fruit and Vegetable Chemistry Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Pasadena, Calif. 91106

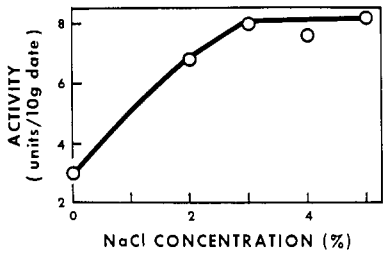

Figure 1. Effect of NaCl concentrations on invertase extraction

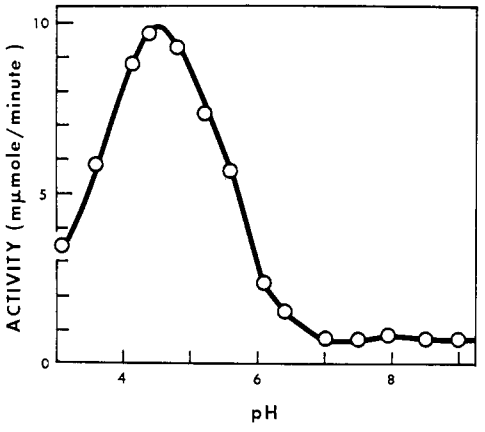

Figure 2. Effect of pH on the invertase activity

The reaction mixture (2.0 ml) consisted of 2.5 × 10^{-3} M sucrose, 0.1 M citrate-phosphate buffer for pH's below 7 or 0.1 M borate buffer for those above 7, and 0.01 unit of invertase

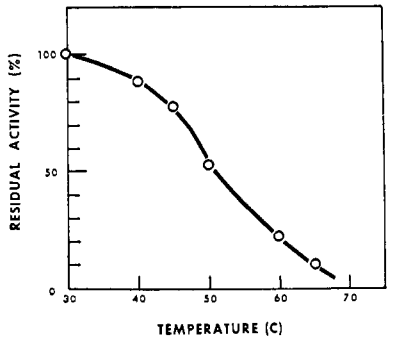

Figure 3. Heat stability of invertase

Invertase solutions, pH 7.0, were treated at various temperatures for 10 min, and the residual activities were determined by the standard procedure

was detected (Figure 2). The enzyme used was partially purified by passing the original salt extract successively through two 2.5 × 45 cm Sephadex G-25 columns. The enzyme was eluted with water.

Both soluble and insoluble fractions were examined with sucrose, raffinose, and melezitose to determine their substrate specificity. The initial rate of reaction was determined by measuring the increase in reducing groups by the procedure of Hobson (1962), and the reaction mixture was examined also by paper chromatographic analysis. As shown in Table II, it was found that both the soluble and insoluble fractions attacked these substrates in a similar manner. The rate of reaction on raffinose was 30 to 32% of that on sucrose. Chromatographic analysis showed that fructose and melibiose were present in the reaction mixture, but galactose was absent. Both fractions also attacked melezitose, yielding

Table I. Solubilization of Insoluble Invertase With Carbowax 4000 and Tween 80

Compounds Added	Solubilized (%)
Control, 0.1 M phosphate buffer, pH 7.0	0
Carbowax 4000, 2%	1.9
4%	1.7
6%	2.2
Tween 80, 2%	0
4%	1.2
6%	1.2

A 3-g portion of insoluble fraction (0.49 unit of invertase per g) was incubated with 10 ml of solutions of Carbowax 4000 or Tween 80 in 0.1 M phosphate buffer, pH 7.0 for 30 min. After centrifugation, the supernatant was assayed by the standard procedure.

Table II. Substrate Specificity[a]

Substrates	Relative Activity: Soluble Invertase, %	Relative Activity: Insoluble Invertase, %
Sucrose	100	100
Raffinose	32	30
Melezitose	1.0	1.6

[a] Activities are expressed relative to rate of sucrose hydrolysis.

Table III. Comparison of Invertase Activity in Four Different Quality of Dates

Grades	Activity (units/date): Insoluble	Soluble	Total
Natural	2.27_b[a] (2.87_b)	8.61_c (10.9_c)	10.88 (13.77)
Waxy	2.19_b (2.88_b)	8.96_c (11.9_d)	11.15 (14.78)
No. 1 Dry	2.00_b (3.03_b)	6.43_b (9.7_b)	8.43 (12.73)
No. 2 Dry	1.50_a (2.54_a)	4.90_a (8.3_a)	6.40 (10.84)

[a] Values with common subscript letters within a column are not statistically different at the 0.05 probability level. Activity expressed as units/10 g dry wt is shown in parentheses.

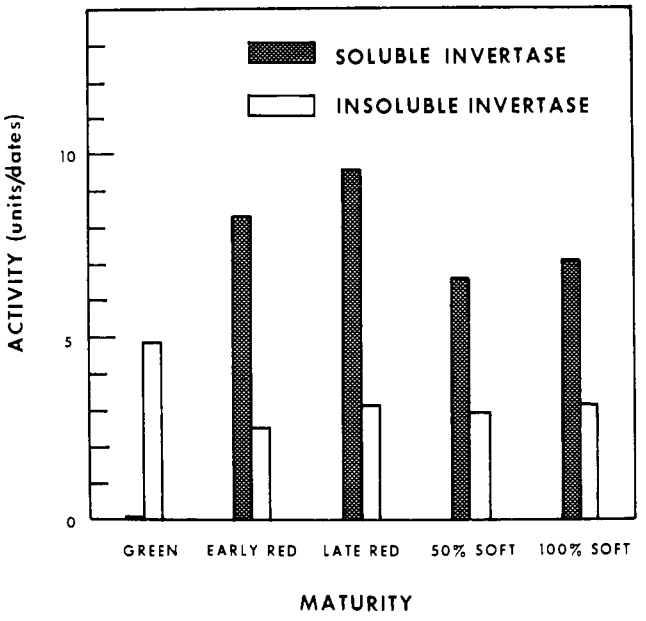

Figure 4. Changes in invertase activity during maturation of Deglet Noor dates. Figures are based on the mean of four determinations

glucose as one of the products, but the rate of hydrolysis was only 1% that of sucrose.

The heat stability of the enzyme is shown in Figure 3. The enzyme was stable at temperatures below 40° C but 90% was inactivated by heating at 65° C for 10 min. Approximately 50% of the original activity was lost when heated at 50° C for 10 min.

Changes in Activity During Maturation. Invertase of dates developed both in the soluble and insoluble fractions with an uneven distribution. At the green stage almost all the activity was found in the insoluble fraction (Figure 4). The activity in the soluble fraction increased sharply during the period that the fruit matured from the green to the early red stages, and reached its maximal level at the late red stage. The activity then decreased slightly as maturity progressed. In contrast to the drastic increase in soluble invertase, the activity of insoluble invertase decreased approximately 50% during the period that the fruit matured from the green to the early red stage. After that the activity remained fairly constant. The ratio of soluble to insoluble invertases changed from about 0.03 in the green stage to 2–3 in matured stages. The fruit contained approximately 12.5 units of total invertase per date at the maximal level.

Activity and Quality of Dates. The differences in invertase activity among the four grades of Deglet Noor dates are shown in Table III. The soft, good quality dates (natural and waxy) had activities higher than those of tough dates (No. 1 and No. 2 dry).

DISCUSSION

The results presented in this paper show that mature dates possess relatively high soluble and insoluble invertase activity. Substantial activity was found only in the insoluble fraction in immature fruit. The activity in the soluble fraction began to develop during the period that the fruit matured from the green to the early red stage. The insoluble invertase decreased about 50% during this stage but remained fairly constant after the early red stage. On the other hand, the soluble invertase increased sharply as the fruit matured from the green to the early red stages. This dramatic increase in invertase activity was very similar to polygalacturonase activity previously observed in dates by Hasegawa *et al.* (1969). The dramatic increase in polygalacturonase activity occurred during the period that the fruit ripened, from the early red to the late red stage. Such a change was observed also on grape invertase (Hawker, 1969a).

Protein-tannin complexes are often formed during the preparation of enzymic extracts from plant tissues (Young, 1965). Treatments with Carbowax or nonionic detergents solubilize most of these complexes (Goldstein and Swain, 1965; Hawker, 1969b). Hawker (1969b) reported that insoluble invertase of grape was solubilized by treating insoluble fractions with Carbowax 4000, Triton 100, or Tween 20. He suggested that the grape invertase previously considered to be insoluble is an artifact which is produced during extraction. Dates contain phenols and tannins (Maier and Metzler, 1965; Rygg, 1946). To determine whether the insoluble invertase of dates is an artifact produced during the extraction, an attempt was made to solubilize insoluble residues by treatment with various concentrations of Carbowax 4000 and Tween 80. The results shown in Table II strongly suggest that the insoluble invertase is not a simple protein-tannin complex. It appears to be bound to the insoluble fraction in such a manner that the enzyme is freely accessible to substrate, and carries out its catalytic function. Thus, in this respect the insoluble invertase of dates is similar to that of several other plants including carrots (Hawker, 1969b; Vaughan and MacDonald, 1967), red beets (Vaughan and MacDonald, 1967) and corn coleoptiles (Hawker, 1969b; Kivilaan *et al.*, 1961).

In some plants the soluble invertase has properties which differ from the insoluble invertase occurring in the same tissues (Sacher, 1966), whereas in other plants the properties of the enzymes are similar (Arnold, 1966; Vaughan and MacDonald, 1967). The results obtained from the substrate specificity study indicate that both soluble and insoluble invertases of dates are similar in these properties.

Sharp increases in activity of several enzymes and in protein synthesis occur in climacteric fruits during the climacteric rise in respiration (Hulme *et al.*, 1963; Hultin and Levine, 1965; Looney and Patterson, 1967). The sharp increase in invertase activity coupled with similar increases in polygalacturonase activity and in protein synthesis (Hasegawa *et al.*, 1969) suggests that dates appear to be a climacteric type. No data on respiration have been reported.

Reducing sugar contents, mainly glucose and fructose, increase gradually throughout development. The increase is more pronounced as the fruit reaches the red stage, and this trend continues through the process of preharvest softening (Coggins and Knapp, 1969). Coggins and Knapp (1969) suggested that the increase in reducing sugars during softening of dates is due to invertase activity enhanced by the loss of membrane system integrity, causing direct contact between enzyme and substrate. The results obtained in this study, however, strongly suggest that the sharp increase in invertase activity is the prime factor for the reducing sugar increase. This suggestion is supported also by the fact that dates which have high reducing sugar contents possess invertase activity higher than that of dates of low reducing sugar contents.

The formation of undesirable tough grades of dates during ripening has been known for many years in the domestic date industry. Various investigators have suggested that developmental processes are terminated by rapid desiccation before the fruits reach full maturity. Our results agree with this postulated view, since tough dates were found to possess lower invertase and polygalacturonase activities, and to contain lower amounts of sugars and proteins than do soft grades.

ACKNOWLEDGMENT

The authors express their sincere gratitude to V. P. Maier for his valuable suggestions in the preparation of this manuscript.

LITERATURE CITED

Arnold, W. N., *Biochem. Biophys. Acta.* **128,** 196 (1966).
Coggins, C. W., Jr., Knapp, J. C. F., *Date Growers' Inst. Ann. Rept.* **46,** 11 (1969).
Cook, J. A., Furr, J. R., *Amer. Soc. Hort. Sci.* **61,** 286 (1953).
Goldstein, J. L., Swain, T., *Phytochemistry* **4,** 185 (1965).
Hasegawa, S., Maier, V. P., Kaszycki, H. P., Crawford, J. K., *J. Food Sci.* **34,** 527 (1969).
Hawker, J. S., *Phytochemistry* **8,** 9 (1969a).
Hawker, J. S., *Phytochemistry* **8,** 337 (1969b).
Hobson, G. E., *Nature* (London) **195,** 804 (1962).
Hulme, A. C., Jones, J. D., Wooltorton, L. S. C., *Proc. Roy. Soc. B.* **158,** 514 (1963).
Hultin, H. O., Levine, A. S., *Arch. Biochem. Biophys.* **101,** 396 (1965).
Kivilaan, A., Beaman, T. C., Bandurski, R. S., *Plant Physiol.* **36,** 605 (1961).
Looney, N. E., Patterson, M. E., *Nature* (London) **214,** 1245 (1967).
Maier, V. P., Metzler, D. M., *Date Growers' Inst. Ann. Rept.* **38,** 6 (1961).
Maier, V. P., Metzler, D. M., *J. Food Sci.* **30,** 80 (1965).
Messer, M., Dahlqvist, A., *Anal. Biochem.* **14,** 376 (1966).
Rygg, G. L., *U.S. Dept. Agr. Tech. Bull.* **910,** 51 pp. (1946).
Sacher, J. A., *Plant Physiol.* **41,** 181 (1966).
Sinclair, W. B., Bartholomew, E. T., Bliss, D. E., *Date Growers' Inst. Ann. Rept.* **19,** 13 (1942).
Vaughan, D., MacDonald, I. R., *Plant Physiol.* **42,** 456 (1967).
Young, R. E., *Arch. Biochem. Biophys.* **111,** 174 (1965).

Received for review March 6, 1970. Accepted May 28, 1970. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.