Invertase (β-Fructofuranosidase) Activity in Three Date Cultivars

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Invertase activity during the last stages of fruit development was determined in three date cultivars. The development of invertase activity occurs in all three cultivars at the beginning of the "Rutab" stage. It was found that maximum invertase activity and its gradual development were different in the three cultivars studied and invertase activity was almost 250 times higher in Khadrawi fruits than in Deglet Noor. These enormous differences in invertase activity between cultivars affect the water activity in the fruits. As a result of this, the rate of water evaporation was lower in fruits of high invertase activity than those with low invertase activity. A significant decrease in the enzyme activity was found at all cultivars during the natural drying of the fruits on the palm or after artificial ripening of "Rutab" fruits. The presence of so many types of dates which differ in texture, color, and taste are discussed in respect to our results.

Dates contain more than 75% sugars on a dry-weight basis, irrespective of the cultivars. Generally, soft-fruit cultivars contain mainly glucose and fructose and little or no sucrose, while fruits of a firm and dry cultivar contain a high percentage of sucrose (Cook and Furr, 1953). The sugar which accumulates in the unripe fruit is sucrose, and only at a later stage it is inverted (Rygg, 1946; Ragab et al., 1956; Coggins and Knapp, 1969). Classification is made between reducing sugar and nonreducing sugar type dates according to the amount of reducing sugars. This classification is not always consistent when intermediate cultivars are considered. The Hallawi fruits are an example of an intermediate cultivar with a tendency toward the reducing-sugars type. Fruits of this cultivar may contain from 0 to 15% of the sugars as sucrose (Cavell, 1947).

Vinson (1907) was the first to point out the role of the enzyme invertase in causing sucrose inversion in dates. More detailed studies on invertase activity were carried out by Hasegawa and Smolensky only in 1970.

The purpose of this study was to measure invertase activity during the last stages of fruit development in several cultivars, representing the spectrum of classification from an extreme reducing sugars cultivar (Khadrawi), to an intermediate cultivar (Hallawi), to a low-reducing-sugars cultivar (Deglet Noor).

MATERIALS AND METHODS

Three cultivars of dates (*Phoenix dactylifera* L.), namely, Khadrawi, Hallawi, and Deglet Noor, used for determinations of invertase activity during ripening, were grown at Kibbutz Sheluhot (Bet She'an Valley). Fruits were sampled during July, August, and September 1974 and sorted according to the following stages: late "Kimri" (green fruits approaching maximum size); "Khalaal" (maximum yellow or red color and firm texture); 50% "Rutab" (light-brown color, softened to the equatorial region); 100% "Rutab" (soft fruits); and "Tamar" (dry fruits).

Frozen samples of ten dates were taken at random, pitted, and sliced into small pieces. The extraction of date invertase was done according to the method of Hasegawa and Smolensky (1970). The enzymatic reaction mixture (5 mL) consisted of 0.1 M acetate buffer, pH 4.5, 2.5 mM sucrose, and an aliquot of dialyzate. The mixture was incubated at 30 °C. One milliliter of each sample was taken at 5-min intervals, after which 3 mL of "Sumner" reagent was added to stop the reaction. The increase in reducing sugars was determined according to the method of Sumner (1925). The optical density was measured at 550 nm using a Perkin Elmer spectrophotometer, Model 139. The control was treated similarly except that the enzyme was heated at 100 °C for 5 min. One unit of invertase was defined as the amount of enzyme which catalyzes the production of 1 μmol of reducing hexoses/min under the above conditions. Moisture content was determined on a 5–10-g sample of pitted dates. The sample was placed on an aluminum dish, weighed, and dried for 24 h in a vacuum oven at 70 °C. The sample was considered dry when the loss of moisture between two successive weighings (4 h apart) was not more than 0.1%. Water activity (a_w) was measured by a Humichek (Sina Ltd., Zurich, Switzerland) on a 10-g sample of pitted dates. The sensor for the instrument used in this study was remounted in a rubber stopper of a 100-mL flask. The a_w values were checked after 24 h at 25 °C.

The rate of water evaporation from the fruits of each cultivar was studied on 150 fruits (from the beginning of "Rutab" stage) from each of three branches of different palms. Thirteen fruits were sampled each time for moisture content.

Table I. Effect of Freezing and Thawing of Fruit on the Activity of Soluble Invertase

Date of sampling	Stage of ripening	Invertase activity, units/g fresh wt: Khadrawi, Unfrozen	Khadrawi, Frozen and thawed	Hallawi, Unfrozen	Hallawi, Frozen and thawed
Sept 29	Late Kimri	0	0	0	0
Sept 29	Khalaal	5	20	0	0
Oct 4	Rutab	9	29	0	5

RESULTS AND DISCUSSION

Invertase activity occurs at the late stages of fruit development; the maximum invertase activity and its gradual development were found to be different in the three cultivars studied (Figure 1). Artificial ripening by freezing and thawing, which brings about the destruction of the fruit structure, seems to bring also the release of the bound enzyme (Table I). For this reason, freezing of fruits was

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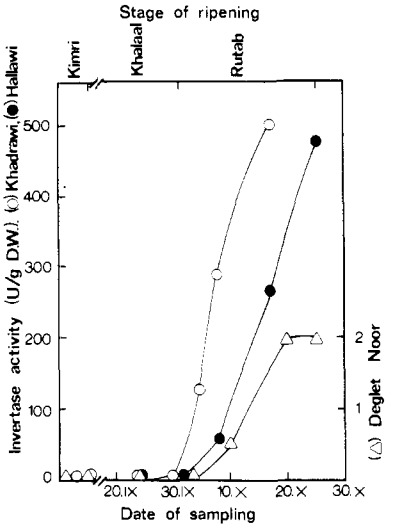

Figure 1. Changes in soluble invertase activity during maturation of three different date cultivars: Khadrawi (○), Hallawi (●), and Deglet-Noor (△).

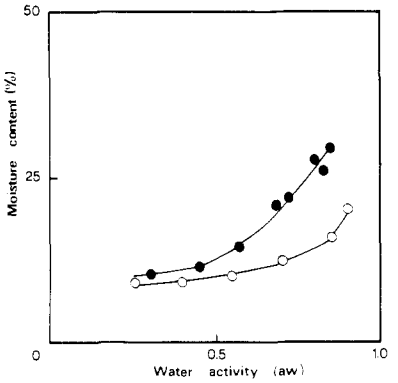

Figure 2. Water desorption isotherm for Khadrawi (●) and Deglet-Noor (○) cultivars.

adopted by us to extract the enzyme. Comparison of the total content of the enzyme shows a basic difference in the different cultivars which seems to be attributable to genetic features. The marked differences in invertase activity among the cultivars reach almost 250-fold (higher in Khadrawi fruits than in Deglet Noor) and may explain the mechanism which contributes to the development of so many kinds of date fruits which differ among them in texture, color, and taste.

Date palm fruits lose a considerable amount of water during the late stage of fruit development. At this stage there is also a sharp increase in sucrose (Rygg, 1946; Coggins and Knapp, 1969; Elmaleh, 1975). At the end of the Khalaal stage the moisture content of normal fruits decreases to 45–55%. The water content in the fruits is influenced by the ratio between the influx and the evaporation of water from the fruit. In cultivars having a high initial invertase activity at the beginning of the "Rutab" stage, the hydrolysis of sucrose is complete and rapid (Kanner, 1967). Similar results were reported by Ragab et al. (1956) which studied the changes in sugars during "Rutab" stage in similar cultivars. This process, which hydrolyzes the sucrose to two molecules, i.e., glucose and fructose, rapidly decreases the water activity of the fruit (Figure 2), which lowers water loss by evaporation. This effect is well demonstrated in Figure 3, which shows the rapid evaporation of water from Deglet Noor dates compared with that from Khadrawi.

Acker (1969), who reviewed the area of enzymatic activity of dehydrated state, presented data showing that the

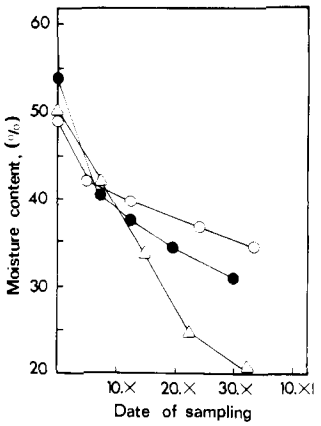

Figure 3. Changes in moisture content during "Rutab" stage in three different date cultivars: Khadrawi (○), Hallawi (●), and Deglet-Noor (△).

rate and extent of hydrolytic and other enzymatic reactions increases as moisture content increases. In fruits with a high invertase activity, a high moisture content is retained in the fruit, which seem to enable other enzymes concerned with fruit softening and darkening to act at better conditions. From the reports of Hasegawa et al. (1972) and Elmaleh (1975) it can be seen that the activity of different enzymes is initiated during the late stages of fruit development. These authors found that a rise in invertase activity occurred earlier than in polygalacturonase and cellulase which soften the fruits, or in polyphenoloxidase which darken the fruit color. The Khadrawi date, because of its high invertase activity at the beginning of the "Rutab" stage, is a reducing type and a soft variety even in hot and dry regions. Hallawi and Deglet Noor dates have a low invertase activity at the begining of the "Rutab" stage. The relative humidity of the air at the beginning of "Rutab" stage can seriously affect the quality of Hallawi dates. Hallawi dates in the Bet She'an Valley seem to have enough water during the "Rutab" stage to enable the invertase to hydrolyze almost all the sucrose to reducing sugars. The humid climate and the complete hydrolysis of sucrose enable the fruit to pass a long "Rutab" stage, during this stage the texture becomes soft and the color brown, like Khadrawi dates. On the other hand, in hot and dry region like the Arava, there are seasons in which Hallawi dates seem to be like the semidry-type dates, and many of the fruits have "white shoulders". The low invertase activity at the beginning of the "Rutab" stage and differences between growing areas could explain the large differences in the amount of reducing sugars in Hallawi dates which were reported by Dowson and Aten (1962).

Our results on invertase activity in Deglet Noor dates are in agreement with those reported by Hasegawa and Smolensky (1970). Compared with other date cultivars the invertase activity of Deglet Noor is very low, even in the late "Rutab" stage. Even in a humid area, this variety behaves like a semidry-type date. However, by artificial ripening of Khalaal-stage Deglet Noor fruits through freezing and thawing, and by holding the fruits under conditions of high humidity and high temperature, the activity of invertase is not critical to the activation of other enzymes which soften and darken the fruits (Kanner, 1967; Elmaleh, 1975). As reported by Hasegawa et al. (1969), Deglet Noor dates contain considerable polygalacturonase and cellulase activities. The polyphenoloxidase activity of Deglet Noor was also found to be very similar to other date cultivars (Elmaleh, 1975). It is not surprising, then, that under optimal artificial ripening conditions Deglet Noor dates could soften and brown like Khadrawi or other

Table II. Changes in Invertase Activity of Three Date Cultivars during the Natural Drying Stage

Date of sampling	Stage of ripening	Invertase activity, units/g of dry wt		
		Khadrawi	Hallawi	Deglet Noor
Oct 4	100% Rutab	372	355	2.4
Oct 17	50% Tamar	164	158	1.2
Oct 25	Tamar	85	112	1.0

Table III. Changes in Invertase Activity during Artificial Ripening of Harvested "Rutab" Fruit

Storage time, days	Invertase activity (units/g dry wt)	
	Khadrawi	Hallawi
0	289	254
7	165	161
14	78	106
14[a]	52	49

[a] Dehydrated at 55 °C for 24 h.

soft-type dates.

During the late "Rutab" to "Tamar" stage, when the dates lose much of their cell wall structure (Coggins et al., 1967) and their water content, invertase activity decreased (Table II). A significant decrease in activity was found also with harvested and stored "Rutab" fruits (Table III). Hasegawa et al. (1969) reported a similar decrease in date polygalacturonase. It is well known that during this period the color of the fruit darkens as a result of enzymatic and nonenzymatic browning (Maier and Schiller, 1961), and both reactions could affect the structure and activity of enzymes (Mathew and Parpia, 1971).

The invertase activity during the "Rutab" stage seems to be a very important factor which contributes also to the development of date taste. As reported by Maier and Schiller (1961) and Maier et al. (1964), the "Maillard reaction" appears to be responsible for the very dark color of dates stored a long time. Nonenzymatic browning in food, mainly from reactions of reducing sugars with amino acids, develop compounds which are responsible for flavors. In Khadrawi and other cultivars which contain high amounts of reducing sugars in the ripe fruits, nonenzymatic browning develop a "caramel"-like taste more rapidly than in Deglet Noor (Kanner, 1967).

To sum up, invertase in dates is a key enzyme which controls the reducing sugars and water content of dates. By this, it seems to affect enzymatic and nonenzymatic reactions responsible for changes in texture, color, and taste of dates ripening naturally on the palm.

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