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LOW TEMPERATURE INDUCTION OF INVERTASE ACTIVITY IN GRAPEFRUIT FLAVEDO TISSUE

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Key Word Index—*Citrus paradisi*; Rutaceae; grapefruit; soluble carbohydrates; cold hardening.

Abstract—Invertase activity increased in the flavedo tissue of 'Marsh' grapefruit (*Citrus paradisi* Macf.) when trees were exposed to cold hardening temperatures and decreased at dehardening temperatures. Invertase activity also increased in the flavedo of detached fruit stored at 5°. Reducing sugar levels paralleled invertase activity while sucrose levels were inversely related to invertase levels. The mechanism by which low temperatures induce invertase activity in grapefruit flavedo tissue was not determined. However, results indicated that a proteinaceous inhibitor, similar to the one found in potato tubers, is not involved in the regulation of invertase activity in flavedo tissue of grapefruit.

INTRODUCTION

Sugars increase in the flavedo (colored portion) of grapefruit peel during the late fall and winter [1, 2]. Initially, sucrose and reducing sugars accumulate simultaneously, but when the daily minimum temperature in groves drops to *ca* 10°, sucrose levels decline with a concomitant increase in the level of reducing sugars [1]. Sucrose levels also decrease and reducing sugar levels increase in the flavedo of detached fruit stored at 5° [3]. Such a pattern of change in sugar composition is suggestive of increased invertase activity in grapefruit peel at low temperatures. Therefore, the relationship between invertase activity and the accumulation of reducing sugars at low temperatures in grapefruit flavedo was investigated.

RESULTS

The seasonal patterns of sugar accumulation and invertase activity in flavedo tissue of grapefruit for the 1981–1982 harvesting season are shown in Fig. 1. As reported previously [1, 2], sucrose increased throughout the fall and winter until mid-January. On the other hand, reducing sugar levels began increasing about mid-November when the mean minimum temperatures dropped below 10°. In mid-February reducing sugar levels declined sharply reflecting the increase in the mean minimum temperature to above 10°. Extractable invertase activity in flavedo tissue was consistently low throughout the harvesting season except for the increase in activity which occurred subsequent to the severe freeze on the

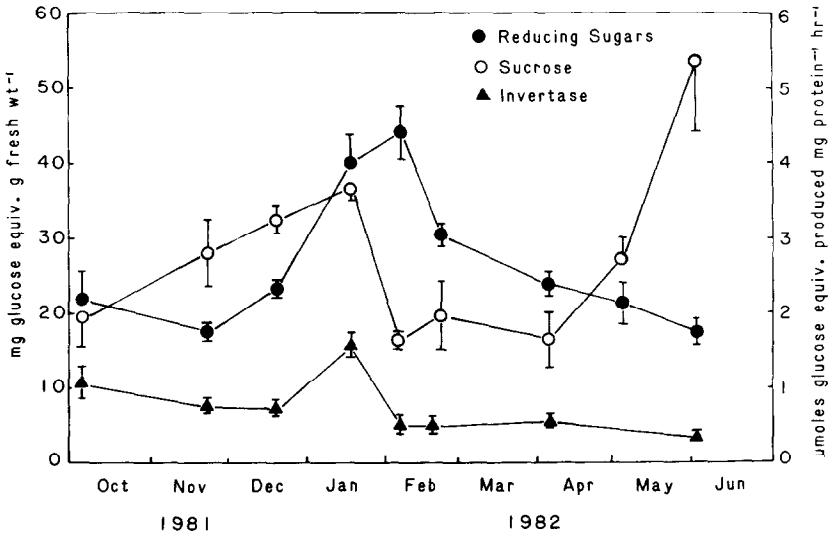

Fig. 1. Seasonal invertase activity, reducing sugar content and sucrose content in grapefruit flavedo tissue. Mean of three samples and S. E.

night of 11 January 1982. The sharp decline in sucrose level opposes the increased invertase activity.

Similar observations were made for fruit from young trees which had been subjected to a low temperature hardening regime in controlled environmental chambers (Fig. 2). Total soluble carbohydrate and reducing sugar contents of flavedo tissue were higher for fruit from hardened trees than for fruit from unhardened trees or trees which had been hardened and subsequently dehardened. In contrast, the sucrose content was lower in the fruit from hardened trees than in the fruit from unhardened or dehardened trees. Invertase activity followed the anticipated pattern, i.e. it was higher in fruit from hardened trees than in fruit from unhardened and dehardened trees.

The change in sugar composition in flavedo tissue of detached fruit stored at 5° also reflects the pattern of invertase activity (Fig. 3). Invertase activity and reducing sugar content increased during storage at 5° while sucrose content decreased. When fruit were transferred from 5° storage to 21° storage for one week, invertase activity declined by 40%. Invertase activity in fruit remaining in 5° storage continued to increase.

The extractability of invertase in the flavedo tissue of grapefruit was authenticated by adding an equal number of units of commercial invertase (Sigma Chemical Co., St. Louis) to samples of hardened and unhardened tissue prior to extraction. Similar amounts of the added enzyme were extracted from both types of tissue (Table 1). However, in contrast to the invertase from grapefruit flavedo which precipitates between 20% and 60% ammonium sulfate saturation, a large proportion of the commercial enzyme remained in solution at 60% ammonium sulfate saturation. To further authenticate the invertase in hardened tissue, extracts of unhardened tissue were added to extracts of hardened tissue. No reduction in invertase activity was observed other than that due to dilution. Finally, extracts from hardened and unhardened tissues were assayed over the pH range of 3.7–8.0. Invertases from both tissues exhibited optimum activity at *ca* pH 4.7.

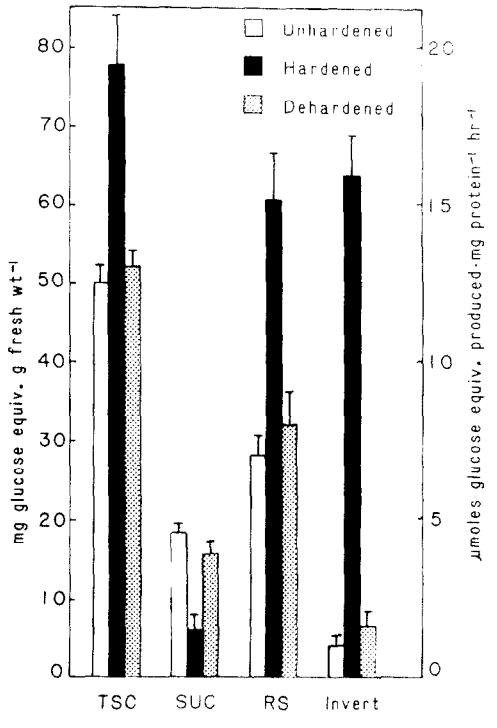

Fig. 2. Total soluble carbohydrates (TSC), sucrose (SUC), reducing sugar (RS) and invertase activity (Invert) in grapefruit flavedo tissue from unhardened, cold hardened and dehardened trees. See experimental procedure for hardening regime. Mean of three samples and S.E.

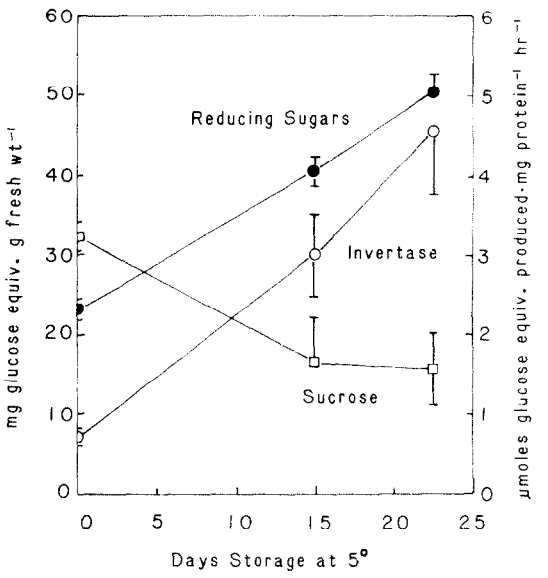

Fig. 3. Invertase activity, sucrose and reducing sugars in flavedo tissue of detached grapefruit stored at 5°. Mean of three samples and S. E.

Table 1. Invertase activity in unhardened and hardened grapefruit flavedo tissue extracted in the presence and absence of commercial invertase

Extraction treatment	Invertase activity (μmol glu. equiv. produced/ml. hr)	
	Unhardened	Hardened
+Commercial Invertase	9.3	28.0
−Commercial Invertase	3.2	22.3
Difference	6.1	5.7

Means of duplicate extractions.

The effect of temperature on *in vitro* invertase activity from grapefruit flavedo is shown in Fig. 4. Activity increased exponentially as the reaction temperature was increased from 5° to 65°. At temperatures above 65°, activity declined sharply.

The rate of sucrose hydrolysis by grapefruit flavedo extracts was linearly related to the protein concentration of the extracts at reaction temperatures of 5°, 25° and 55° (Fig. 5).

DISCUSSION

This study indicated that low temperature-induced invertase activity is primarily responsible for the accumu-

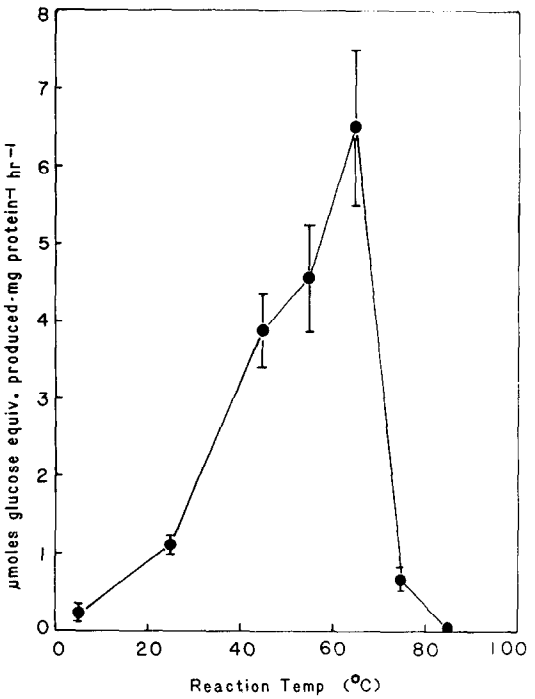

Fig. 4. Effect of reaction temperature on *in vitro* invertase activity from grapefruit flavedo tissue. Mean of three samples and S. E.

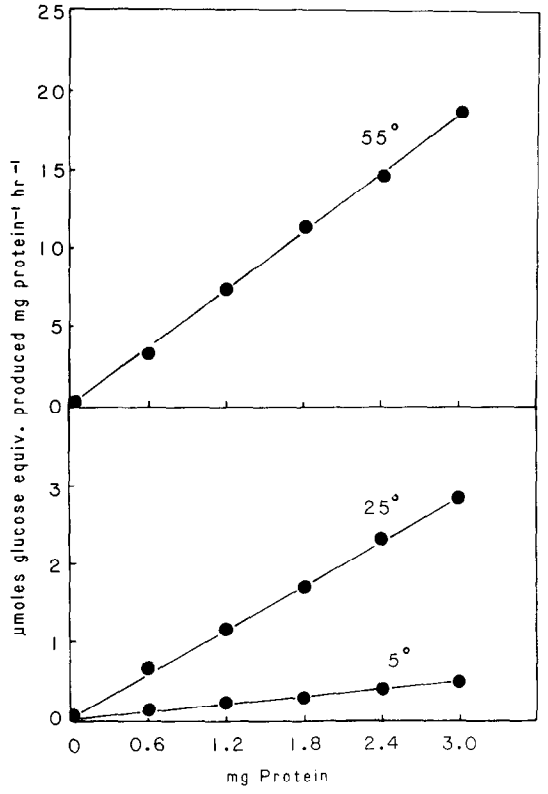

Fig. 5. Effect of protein concentration in reaction on sucrose hydrolysis at 5°, 25° and 55°. Note changes in value on ordinate.

lation of reducing sugars in grapefruit flavedo tissue. It does not, however, rule out other factors which affect accumulation, such as a reduction in the utilization of hexoses at low temperatures. Similar conclusions were reached for the accumulation of reducing sugars in cold stored potato tubers [4].

The mechanism of increased invertase activity at low temperatures in grapefruit could not be ascertained from this study, but several mechanisms have been delineated for other plant tissues. In low temperature stored potato tubers, the increased invertase activity is brought about by a reduction in the activity of a proteinaceous inhibitor which binds irreversibly with the enzyme [5–7]. Thus, when tubers are stored at 18° high inhibitor and low invertase activities are found, whereas the reverse is observed at 4°. Invertase activity in developing maize endosperm is also controlled by a proteinaceous inactivator which apparently is not regulated by temperature [8]. Inhibitors of invertase have been reported for other plant tissues [9, 10].

Attempts to demonstrate an inhibitor of invertase in grapefruit flavedo tissue were unsuccessful. Foaming the extracts by blending or bubbling with nitrogen, treatments which are effective in dissociating potato tuber enzyme and inhibitor [6, 11], did not increase the specific activity of the enzyme. Invertase activity in grapefruit flavedo extracts was linear with protein concentration and mixing extracts lacking invertase activity with those exhibiting activity did not reduce activity other than from dilution. However, Ewing and McAdoo [7] point out that an inhibitor, which forms an essentially non-dissociable complex with an enzyme, ordinarily would not affect the linearity of the reaction. The failure to demonstrate the presence of an inhibitor of invertase in grapefruit flavedo extracts with methods employed for potato tubers does not rule out that one exists. Nevertheless, invertase activity can be regulated in plant tissues in other ways.

The *in vitro* activity of invertase from grapefruit exhibited a single temperature optimum at *ca* 65°. In contrast, invertase from mango fruit exhibits two temperature optima, one near 0° and the other near 40° [12]. The two optima could represent the activity of different invertase isoenzymes. Isoenzymes of invertase are widely distributed among tissues of several plant species [13–15]. We are investigating the possibility that temperature-sensitive isoenzymes exist in grapefruit flavedo.

The role of invertase in grapefruit flavedo at low temperatures is not known. High invertase activity is generally detected in most plant tissues at stages when the growth rate is high [16–18]. Thus, it is linked to energy production and the provision of carbon skeletons for the synthesis of various cellular constituents. In storage organs, such as potato tubers, invertase activity increases during sprouting and low temperature storage [5]. The increased activity during sprouting can be attributed to the initiation of growth processes, but the increased activity at low temperatures has not been well explained. In grapefruit flavedo, reducing sugars accumulate prior to the development of the fruit's resistance to chilling injury but their role in the resistance mechanism has not been delineated [1–3]. The present study indicates that reducing sugars accumulate as a consequence of increased invertase activity induced by low temperatures. If, indeed, reducing sugars have a role in the mechanism of resistance to chilling injury, modulation of invertase activity becomes increasingly important.

EXPERIMENTAL

Plant material. The grapefruit (*Citrus paradisi* Macf. 'Marsh') utilized for all expts, except the controlled low temp. hardening expt, were harvested from field research plots. For the controlled low temp. hardening expt, young trees were cultured and hardened as previously described [3] except for the temp. regimes imposed. Initially, the trees were maintained at 25°. The temp. was first decreased to 15° for two weeks and then to 5° for another two weeks. Trees were dehardened by increasing the temp. from 5° to 25° for two weeks.

Tissue preparation. Analyses were made on individual fruit. The flavedo of the fruit was removed in 5 mm wide strips, rinsed generously in deionized H_2O and blotted with cheesecloth before being cut into 1 cm pieces. Invertase was extracted from 10 g subsamples and the remainder of the tissue was stored in polyethylene bags at −22°.

Enzyme preparation. Tissue (10 g) was mixed with 20 ml 10 mM KPi buffer, pH 7, and 100 mg Polyclar AT [poly(vinyl polypyrrolidone)] and homogenized for 15 min with a Sorvall Omnimixer at 75% max. speed, and for 1 min with a Polytron homogenizer, also at 75% max. speed. The homogenate was filtered through four layers of cheesecloth, and centrifuged for 20 min at 26 000 *g*. The supernatant was fractionated with $(NH_4)_2SO_4$ (in a satd soln at 4°) and the fraction ppting between 20 and 60% satn was collected by centrifugation at 12 000 *g* for 15 min. The ppt was dissolved in 2 ml 10 mM KPi buffer, pH 7, and dialysed for 18 hr against the same buffer. All extraction procedures were carried out at 0–4°.

Enzyme assay. Invertase was assayed by a modification of the procedure of ref. [19]. The reaction mixture contained 73 μmol sucrose in 80 mM NaOAc buffer, pH 4.7, in a total vol. of 0.45 ml. After 5 min equilibration in a water bath at 55°, unless otherwise noted, the reaction was initiated by the addition of 50 μl of extract. The reaction was terminated after 15–30 min (depending on activity of enzyme) by the addition of 0.5 ml Nelson–Somogyi copper reagent [20]. Tubes with substrate but lacking extract were incubated along with the reaction tubes and extract was added following the addition of the Nelson–Somogyi copper reagent. The tubes were capped, boiled for 20 min, cooled and mixed with 0.5 ml of arseno–molybdate reagent [20] and 6 ml H_2O. The *A* was measured at 540 nm and compared to appropriate concns of a glucose standard.

Protein assay. Protein content of the extracts was determined by the Bio-Rad procedure [21] utilizing bovine serum albumin as a standard.

Sugar analyses. Subsamples of 5 g of flavedo were extracted with boiling 80% EtOH, washed with petrol and treated with ion exchange resins as previously described [2]. Total soluble carbohydrates were determined by the anthrone method [22] and reducing sugars by the Nelson test [20]. In addition, samples of the resin-treated EtOH extracts were silylated with Tri-Sil (Pierce Chemical Co., Rockford, IL) and sugars were determined by GC [23].

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