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Carbohydrate Metabolism As Related to High-Temperature Conditioning and Peel Disorders Occurring during Storage of Citrus Fruit

NÉLY HOLLAND,† HILARY C. MENEZES,† AND MARÍA T. LAFUENTE*,§

Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Apartado de Correos 73, 46100 Burjassot, Valencia, Spain, and Departamento de Tecnologia de Alimentos, FEA—Universidade Estadual de Campinas (UNICAMP), Caixa Postal 6121, CEP 13083-970, Campinas, Brazil

The aim of this research was to understand the involvement of the carbohydrate metabolism in physiological disorders occurring during the postharvest storage of citrus fruit. These disorders, manifested in the rind, depreciate fruit quality and often originate important losses. There has been increasing interest in the use of nonharmful treatments, such as high-temperature conditioning, to avoid citrus peel damage during fruit storage at low temperature in chilling-sensitive cultivars, but their influence in postharvest disorders occurring at nonchilling temperatures and the mechanisms related to them are poorly understood. The data obtained showed that heat conditioning (3 days/37 °C) increases the chilling tolerance of cv. Navelate fruit and favored sucrose, but not hexoses, accumulation and its maintenance after the fruit was transferred to low temperature. This effect was related to heat-induced increase in the activities of the sucrose-synthesizing enzymes sucrose phosphate synthase (SPS) and sucrose synthase (SS). Furthermore, sucrose levels and the activities of both enzymes were higher in cv. Pinalate oranges, a chilling-tolerant spontaneous abscisic acid deficient mutant of Navelate. In contrast, carbohydrates appeared not to be involved in the susceptibility of oranges to rind staining, a physiological disorder different from chilling injury, which mainly occurred at a nonchilling temperature (12 °C) and was not reduced by heat conditioning. The effect of low temperature in SS and SPS activities was less than that of high temperature, which might be related to the lower changes occurring in sucrose during fruit storage at 2 °C.

KEYWORDS: Abscisic acid (ABA); citrus; chilling and nonchilling physiological disorders; ethylene; heat conditioning; soluble carbohydrates; starch; sucrose phosphate synthase; sucrose synthase

INTRODUCTION

Postharvest peel damage of citrus fruit may be caused by different stress conditions, including chilling (*1*, *2*), water stress (*3*), and alteration of internal gas levels during fruit storage, originating very important economical losses because of quality loss (*4*). The orange fruit cultivar Navelate (*Citrus sinensis* L. Osbeck) develops chilling injury (CI) after prolonged storage at 2 °C, which is manifested as brown nondepressed areas in the flavedo (the outer colored part of the peel) (*5*). In addition, this citrus cultivar is prone to develop another postharvest physiological disorder after being exposed for short periods to nonchilling temperatures (>10 °C), called "rind staining" (RS). This disorder appears to be related to water stress (*6*). Extensive collapsed and dried peel areas of part of the albedo (inner part of the peel) and of the flavedo, which become dark brown as time progresses, are the main symptom of RS, which, therefore, does not resemble CI. Recently, a spontaneous mutant of this citrus cultivar, the orange cv. Pinalate, has been characterized (*7*), which is yellow, deficient in abscisic acid (ABA), tolerant to chilling, and more susceptible to RS than Navelate oranges (*5*). Consequently, this mutant is a useful tool to study the mechanisms involved in the tolerance of citrus fruit to both physiological disorders.

The use of nonharmful treatments to reduce the incidence of these disorders is desirable. During recent years there has been increasing interest in the use of high-temperature conditioning treatments to protect a number of vegetables and fruit against CI (*8*). However, the influence of heat conditioning in the development of other postharvest disorders that depreciate fruit quality and the mechanisms related to them are poorly understood.

Sugars are ubiquitous osmolytes in plants and may stabilize proteins from thermal stress denaturation through their effect on water structure and the degree of hydrophobic interactions exerted between biomolecules. Alternatively, sugars may provide greater stability to chemical reactions and biomembranes under desiccation and other environmental stresses because of their ability to form metastable "glassy" states, characterized

* Author to whom correspondence should be addressed (e-mail mtlafuente@iata.csic.es; telephone 34-963900022; fax 34-963636301).
† Universidade Estadual de Campinas.
§ CSIC.

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by high viscosity (*9*). The accumulation of soluble sugars and starch has been related to the ability of plants to develop chilling tolerance (*10*), and the effect of high temperature preventing sucrose decline during cold storage of cv. Fortune mandarins has been reported (*11*). However, the influence of high temperature on the activity of the enzymes of carbohydrate metabolism and how this affects the postharvest behavior of citrus fruit stored at chilling and nonchilling temperatures have not yet been reported. In fruit, the accumulation of sucrose depends on both sucrose phosphatase synthase (SPS; EC 2.4.1.14) and sucrose synthase (SS; EC 2.4.1.13) (*12*, *13*), and both enzymes can be affected by high- and low-temperature stress (*14*−*17*). There is also a lack of information on the influence of ABA, a plant hormone playing a protective role against dehydration, on both enzymes and on sugar accumulation in the peel of citrus fruit, which has been shown to differ among plant systems (*18*, *19*).

The aim of this work was to study the effect of heat conditioning on changes in the carbohydrate metabolism of citrus fruit and its influence in postharvest rind disorders occurring at chilling and nonchilling temperatures. Changes in carbohydrates, as well as in SS and SPS activities, were evaluated. Fruit samples of the cultivar Navelate and its ABA-deficient mutant (Pinalate) were selected because of the different susceptibilities shown to both physiological disorders. In addition, Pinalate fruit provided an excellent material to study whether ABA may influence the cold- and heat-induced changes in carbohydrate metabolism of citrus fruit.

MATERIALS AND METHODS

Plant Material and Postharvest Treatments. Navelate and Pinalate oranges (*C. sinensis* [L.] Osbeck) were harvested at the late maturity stage from trees grafted onto Citrange carrizo rootstock, from the same orchard in Murcia, Spain, and immediately delivered to the laboratory. Pinalate oranges were yellow, whereas Navelate oranges showed the characteristic orange color of mature citrus fruit. Fruit samples from both cultivars were randomly divided into two batches. Samples from the first batch were conditioned at 37 °C and 90−95% relative humidity (RH) for 3 days and then subdivided into two groups, which were stored at 80−90% RH and either 2 or 12 °C for 88 days. The second batch was subdivided into two groups, which were stored immediately (nonconditioned) in the same way as the conditioned fruit. For each temperature regime and cultivar, three replicates, each containing 10 pieces of fruit, were used to determine the CI and RS indices and three replicates of 10 pieces of fruit per storage period were used to analyze the carbohydrate content and the activities of the enzymes SS and SPS. Flavedo tissue was separated from the total surface of the fruit, frozen in liquid nitrogen, and kept at −80 °C for later sugar and starch content analyses and for the enzyme assays.

Estimation of Chilling Injury and Rind-Staining Indices. The CI and RS indices were visually estimated weekly during holding of conditioned and nonconditioned fruit at 2 and 12 °C. Because RS symptoms do not resemble those of CI, the severity of both physiological disorders was evaluated independently. Rind-stained fruit showed collapsed areas, which became dark brown with time, whereas CI was manifested as brown nondepressed areas of the flavedo. A rating scale from 0 (no damage) to 3 (severe damage) was used to estimate the CI and RS indices. Both indices were determined by adding the products of the number of fruit in each category multiplied by its score and then dividing the total obtained by the number of pieces of fruit evaluated (*20*). The results were the means ± standard error of the mean (SEM) of three replicate samples containing 10 pieces of fruit each.

Sugar and Starch Analyses. Sugars and starch from flavedo tissue were analyzed as previously described (*21*). Sugars were extracted with 80% boiling ethanol (*22*), and 2.1% raffinose was added as an internal standard after the ethanol extracts had been filtered. The ethanol was evaporated off and the residue dissolved in water. This solution was purified by passing through a C-18 Elud Bond cartridge from Varian (Harbor City, CA) and then filtered through a Millipore HV-4 filter (pore size = 0.45 μm) (Ibérica, S.A., Barcelona, Spain) before HPLC analysis. A 300 × 7.8 mm Phenomenex column packed with a Rezex sulfonated polystyrene resin and a Waters 410 refractive index detector (Waters, Franklin, MA) were used for HPLC analyses. The sugars were eluted at 85 °C in 20 min using water as the mobile phase at a flow rate of 0.6 mL min^{-1}. The results were the mean ± SEM of three replicate samples of 10 pieces of fruit each.

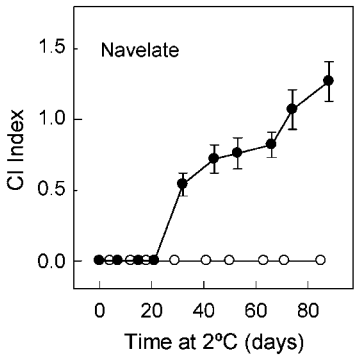

Figure 1. CI index of Navelate oranges stored immediately after harvest (●) or after 3 days of conditioning at 37 °C and 90–95% RH (○). Values are the means ± SEM of three replicate samples containing 30 fruit.

Starch was extracted from the dried insoluble residue obtained after ethanol extraction according to the method of Lafta and Lorenzen (*23*). The dried residue was rehydrated in water, heated for 1 h at 90 °C, and incubated with an amyloglucosidase solution (10 units mL^{-1}, 20 mM NaF, 100 mM acetate buffer, pH 4.5) for 48 h at 40 °C, and the glucose released was determined colorimetrically in a glucose oxidase-coupled reaction. The starch content was expressed as milligrams of glucose released from starch per gram of dry flavedo tissue.

Sucrose Synthase and Sucrose Phosphatase Synthase Assays. The activities of the enzymes SPS and SS, assayed in the synthesis direction, were determined from the same extract. The enzymes were extracted from 1 g of frozen flavedo samples as previously described by Holland et al. (*21*). The frozen tissue was ground under liquid nitrogen in a chilled mortar with 5 mL of 50 mM MOPS/NaOH (pH 7.5) chilled buffer containing 10 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, and 0.1% (v/v) Triton X-100, the homogenate was centrifuged, and the supernatant was desalted using a Sephadex G-25 (1 × 5 cm) column from Amersham Pharmacia Biotech AB (Uppsala, Sweden) equilibrated with extraction buffer minus Triton X-100 (*16*). The activities of both enzymes were determined according to the method of Guy et al. (*16*) except that quantification of sugar formation was measured using the phenol−sulfuric method (*24*). For SPS, the reaction mixture contained 10 mM UDP-glucose, 10 mM fructose 6-phosphate, and 40 mM glucose 6-phosphate in 50 mM MOPS/NaOH (pH 7.5) buffer. The same reaction mixture was used for SS but containing 10 mM fructose instead of the mixture of fructose 6-phosphate and glucose 6-phosphate. The soluble protein concentration in the extracts was measured according to the method of Bradford (*25*).

Statistics. The values are the means ± SEM of three replicate samples. The data were analyzed using ANOVA (*F* test, ≤0.05).

RESULTS

Influence of High-Temperature Conditioning on the Incidence of CI and RS in Navelate and Pinalate Oranges. Nonconditioned Navelate oranges showed CI after 30 days of storage at 2 °C. Thereafter, the CI index increased continuously up to 88 days of exposure at this temperature (**Figure 1**). No chilling symptoms were observed in the flavedo of fruit conditioned for 3 days at 37 °C and then held at 2 °C (**Figure 1**) or in conditioned and nonconditioned fruit stored at 12 °C (data not shown). The mutant Pinalate did not show CI symptoms but was more susceptible to RS than the cultivar Navelate.

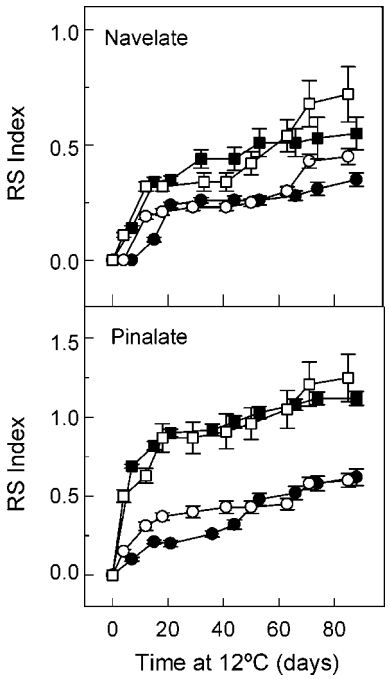

Figure 2. Rind staining index of Navelate and Pinalate oranges stored immediately after harvest (nonconditioned fruit) or after 3 days of conditioning at 37 °C and 90–95% RH (conditioned fruit) with subsequent chilled (2 °C) or nonchilled (12 °C) storage. Oranges were exposed to the following treatments: (1) nonconditioned fruit stored at 2 °C (●); (2) conditioned fruit stored at 2 °C (○); (3) nonconditioned fruit stored at 12 °C (■); (4) conditioned fruit stored at 12 °C (□). Values are the means ± SEM of three replicate samples containing 30 fruit.

RS symptoms were detected in both cultivars at 2 and 12 °C, although it was very low at 2 °C (**Figure 2**). The RS index of Pinalate fruit increased much more rapidly than in Navelate fruit at 12 °C. After 10 days of storage at this temperature, the RS index of nonconditioned Pinalate fruit was 0.69, whereas that of Navelate oranges was 0.14. At the end of the storage period, the RS index of Pinalate was double that of Navelate fruit. Heating did not affect RS in fruit held at 12 °C. However, conditioning the fruit at high temperature significantly ($P \leq 0.05$) accelerated the appearance of RS at 2 °C, this effect being more marked in Pinalate than in Navelate oranges. No difference in the incidence of RS was observed between conditioned and nonconditioned fruit after prolonged cold storage.

Changes in Soluble Sugar and Starch Contents. Sucrose was the least abundant soluble sugar in the flavedo of both cultivars, but showed the most relevant changes under the different thermal conditions imposed on the fruit (**Figure 3**). The sucrose content in the flavedo of Pinalate oranges was double that of the Navelate oranges, whereas the levels of reducing sugars were similar (**Figure 3**).

The sucrose content decreased considerably in nonconditioned Navelate and Pinalate fruit held at 12 °C, the decline being faster in the Pinalate fruit. At 2 °C, the sucrose levels increased during the first 30 days in the Navelate fruit and subsequently declined to reach the initial levels. In Pinalate oranges, a continuous decline in this soluble sugar was observed after 14 days at this temperature. In general, the sucrose levels were significantly lower ($P \leq 0.05$) in nonconditioned Navelate and Pinalate oranges held at 12 °C than in oranges held at 2 °C. However, after 88 days no differences were found between the Pinalate fruit kept at these temperatures. Conditioning the fruit for 3 days at 37 °C significantly increased sucrose levels from 3.6 to 7.3 mg g^{-1} of fresh weight (FW) and from 7.3 to 14.2 mg g^{-1} of FW in Navelate and Pinalate fruit, respectively. Heat conditioning also favored the increase in sucrose occurring in Navelate oranges during holding of the fruit at 2 °C, and by 45 days of storage, the sucrose content of conditioned fruit was 3 times that of nonconditioned fruit. Conditioned Pinalate fruit showed a continuous decrease in sucrose levels when they were transferred to 2 °C, but the levels were always much higher than those of nonconditioned fruit held at this temperature. By contrast, no difference was found between conditioned and nonconditioned fruit of the selected cultivars after 14 days of storage at 12 °C. Fruit kept at this temperature showed, in general, the lowest sucrose levels. Conditioning fruit of both cultivars induced fewer relevant changes in the hexose levels (**Figure 3**). By the end of the storage period the hexose levels of fruit maintained at 2 °C were significantly higher than those of fruit kept at 12 °C. It can also be noted that the glucose and fructose contents of Navelate oranges decreased more quickly ($P \leq 0.05$) in conditioned than in nonconditioned fruit stored at 2 and 12 °C.

The starch content in the flavedo of freshly harvested Pinalate fruit was ~2.5 times that of Navelate fruit. It sharply decreased in both cultivars when the fruit was held at 2 or 12 °C, and no significant difference was found between fruit samples stored at either temperature (**Figure 4**). The heat conditioning treatment originated a slight reduction in starch levels in Navelate fruit but an increase in the mutant Pinalate. The starch contents of the conditioned Navelate and Pinalate fruit stored for 30 days at 2 °C were considerably higher than those of conditioned fruit held at 12 °C and those of nonconditioned oranges held at 2 or 12 °C, but no difference was found after prolonged storage (88 days) (**Figure 4**).

Changes in Sucrose Synthase and Sucrose Phosphatase Synthase Activities. Because heat conditioning increased sucrose levels in the flavedo of Pinalate and Navelate oranges and avoided their decrease during holding of the fruit at 2 °C, changes in SS, assayed in the sucrose synthesis direction, and in SPS in conditioned and nonconditioned fruit of both cultivars held at this temperature were examined. In concordance with the sucrose concentrations, extractable activities of both enzymes were significantly higher ($P \leq 0.05$) in Pinalate than in Navelate oranges at harvest (**Figure 5**, 0 days of storage**)**. Activities of both enzymes markedly increased ($P \leq 0.05$) during the heat conditioning treatment in Navelate fruit. After 3 days of conditioning, both SPS and SS increased 1.8-fold in this citrus cultivar. Heat conditioning also favored the induction of both enzymes in Pinalate fruit but to a lower extent than in Navelate. Thus, a 1.4- and 1.3-fold increases in SPS and SS were found, respectively, in Pinalate fruit. Heat-conditioned Navelate and Pinalate fruit stored at low temperature had, in general, a higher SS activity than nonconditioned fruit. A similar trend was found in the SPS activity in Navelate oranges, but no relevant differences were found in the activity of this enzyme between conditioned and nonconditioned fruit in the cultivar Pinalate.

DISCUSSION

Sugars have been reported to play a protective role in plants against stress conditions (*9*). In citrus fruit they have been related to different physiological processes, including drought (*26*), thermal stress (*27*), maturity (*21*, *28*), and color changes (*29*−*31*). Our results, showing that the sucrose and starch contents are considerably lower in Navelate oranges than in its mutant Pinalate, whereas hexose levels are similar, and that Pinalate oranges are more likely to develop RS than Navelate fruit, indicate that the carbohydrate content is not a limiting factor in

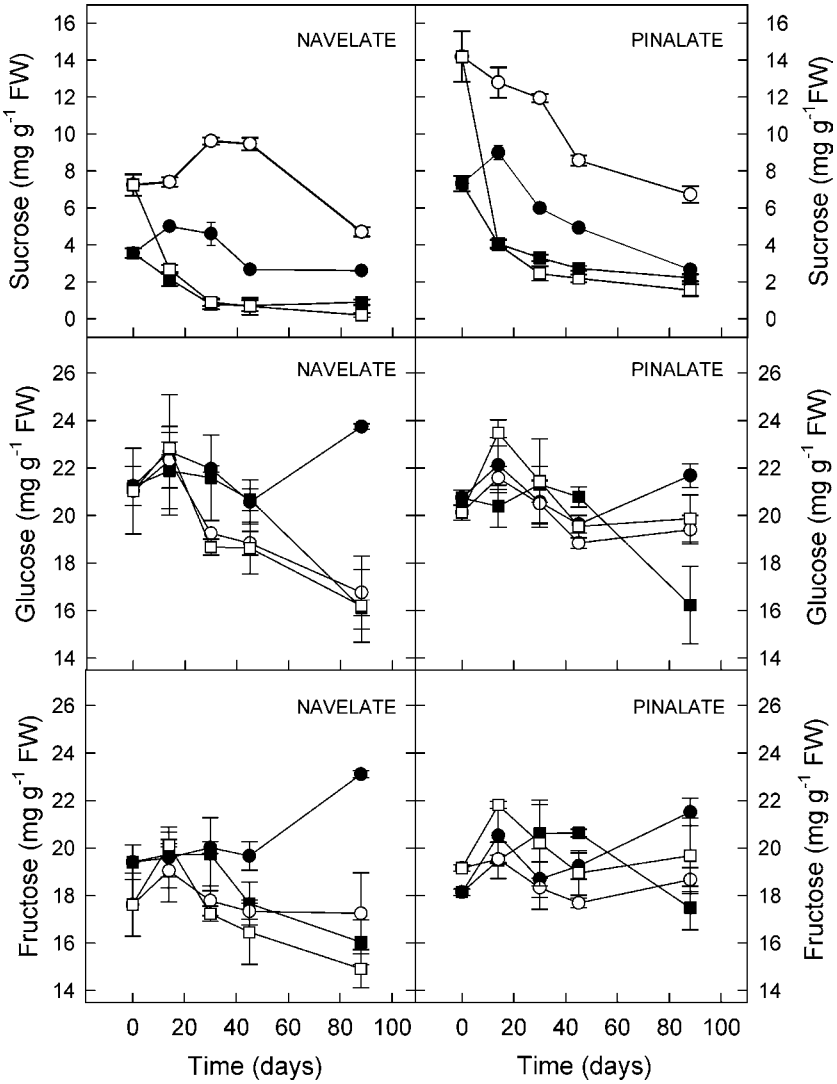

Figure 3. Sucrose, glucose, and fructose contents in the flavedo of Navelate and Pinalate oranges stored immediately after harvest (nonconditioned fruit) or after 3 days of conditioning at 37 °C and 90–95% RH (conditioned fruit), with subsequent chilled (2 °C) or nonchilled (12 °C) storage. Oranges were exposed to the following treatments: (1) nonconditioned fruit stored at 2 °C (●); (2) conditioned fruit stored at 2 °C (○); (3) nonconditioned fruit stored at 12 °C (■); (4) conditioned fruit stored at 12 °C (□). Values are the means ± SEM of three replicate samples containing 10 fruit.

the tolerance of a specific citrus cultivar against this water stress-related physiological disorder (*5*). It is well-known that ABA mediates drought-induced stomatal closure and plays a role in minimizing water loss in plant systems and that water stress leads to an increase in soluble sugar in citrus fruit attached to the tree (*26*, *32*). Therefore, our results showing a higher sucrose content in the flavedo of Pinalate oranges attached to the tree may well indicate that increased sucrose levels in this cultivar represent a fruit defense mechanism, balancing the deficiency in ABA to better support field environmental conditions favoring its dehydration. However, as indicated above, the increased sucrose levels were not sufficient to protect harvested Pinalate fruit against RS, which mainly occurred during the storage of conditioned and nonconditioned fruit at 12 °C (**Figure 2**). This temperature originated a higher decline in sucrose than the lower storage temperature (2 °C) (**Figure 3**) despite inducing a higher rate of water loss (*5*). Because the oranges were detached from the parent plant, this event may well be related to the higher respiratory rate occurring in citrus fruit stored at 12 °C (*11*).

Interestingly, sucrose appears to have an effect on color change (*31*), but mature fruit of this spontaneous yellow mutant, which shows a slower rate of color break and is not able to develop the characteristic color of citrus orange fruit (*7*), presented higher sucrose levels than fruit of its parental Navelate. Whether the chloroplast to chromoplast conversion in the epicarp of these fruit is affected or not by sucrose deserves further investigation. It is also interesting that the most important changes in glucose and fructose during the maturation of cv. Fortune mandarins occurred at color break (*21*) in parallel with the shift in ABA (*20*). The results presented here show that the reducing sugar contents in mature Navelate fruit and in its ABA-deficient mutant are similar (**Figure 3**). Therefore, it seems that ABA is not a limiting factor for the increase in glucose or fructose during maturation of citrus fruit.

The accumulation of sugars has been related to the cold acclimation of different plants (*16*, *33*). A higher tolerance to chilling was found in Pinalate than in Navelate oranges. From this result, we cannot rule out the possibility that sucrose may contribute in part to the higher tolerance of Pinalate fruit to CI. However, it should be taken into consideration that other citrus cultivars, such as Fortune mandarins, showing more susceptibility to chilling than Navelate fruit, presented higher sucrose contents than Pinalate oranges and similar hexose levels (*21*).

The beneficial effect of heat conditioning on the quality of fruits and vegetables during their postharvest life has been extensively shown (*8*, *20*, *34*). Our results demonstrated that

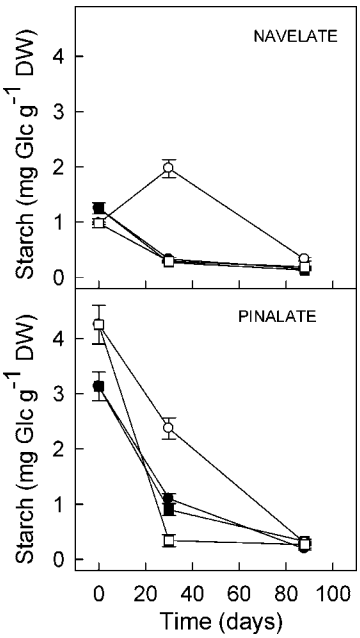

Figure 4. Starch contents in the flavedo of Navelate and Pinalate fruit stored immediately after harvest (nonconditioned fruit) or after 3 days of conditioning at 37 °C and 90–95% RH (conditioned fruit), with subsequent chilled (2 °C) or nonchilled (12 °C) storage. Oranges were exposed to the following treatments: (1) nonconditioned fruit stored at 2 °C (●); (2) conditioned fruit stored at 2 °C (○); (3) nonconditioned fruit stored at 12 °C (■); (4) conditioned fruit stored at 12 °C (□). Values are the means ± SEM of three replicate samples containing 10 fruit.

curing (3 days at 37 °C) reduced the cold-induced peel damage during exposure of Navelate fruit to a chilling temperature (2 °C). Furthermore, we have shown that despite the high efficacy of this postharvest heat treatment in reducing CI in this (**Figure 1**) and other citrus cultivars (*20*), it did not prevent RS, which mostly occurred at the nonchilling temperature (12 °C) in Pinalate oranges (**Figure 2**). Heat conditioning significantly increased sucrose levels in Pinalate and Navelate oranges but exerted little effect on the hexose contents. In addition, heated fruit of both cultivars kept at 2 °C presented higher sucrose levels than the unheated fruit, despite no differences being found during the holding of the fruit at 12 °C. High temperature (37 °C) sharply increased the fruit respiratory rate in citrus fruit (*11*). We could therefore have expected a higher consumption of carbohydrate reserves for respiration instead of increased carbohydrate levels. In light of our results we hypothesized that enzymes involved in sucrose synthesis might be activated in response to heating in these citrus cultivars, favoring sucrose accumulation, despite the heat-induced increase in fruit respiration. As the sucrose content was maintained higher in conditioned fruit kept at 2 °C, we studied changes in the activities of the enzymes SPS and SS (in the sucrose synthesis direction) in Navelate and Pinalate oranges exposed to such thermal conditions. We concentrated on these enzymes because they may be altered in response to low and high temperatures in plant tissues (*16*, *17*, *35*, *36*).

In agreement with the differences found in the sucrose contents of Navelate fruit and in those of its mutant Pinalate, the activity of the sucrose synthetic enzymes examined here were higher in the mutant at 0 days of storage (**Figure 5**). Therefore, both enzymes appear to be involved in increased sucrose accumulation in the flavedo of citrus fruit. This is in agreement with other reports showing that the accumulation of this soluble sugar in fruit depends on both SPS and SS synthesizing enzymes (*12*, *13*). Aung et al. found that glucose and fructose decreased while sucrose increased in lemons in response to a 55 °C heat shock of 3 min and suggested that the heat-induced increase in sucrose might be derived from both hexoses (*37*). The facts that the higher sucrose contents in Pinalate fruit as compared to those of Navelate fruit were not accompanied by markedly lower hexose or starch contents in the mutant and that the starch reserve was very low suggest that alternative sources of carbon for sucrose synthesis may have been used. In this respect, Vu et al. showed that cv. Hamlin sweet orange cell cultures efficiently used glycerol as a source of carbon for synthesizing sugars through enhanced SPS and SS activities (*38*). The lack of correlation between both events could also be related to other factors, such as the differential cellular compartmentalization of these enzymes and sucrose. Alternatively, the differences found in sucrose may arise from its translocation into the fruit from the plant (*39*).

Considerable interest has centered on the role of SPS and SS in the regulation of sucrose synthesis in thermally stressed plants. The activity of both enzymes was found to increase in wheat plants after cold shock, the rise being larger in the more cold-tolerant cultivars (*15*). Increases in sucrose and SPS steady-state mRNA levels and SPS activity, due to higher SPS protein levels, were also found in kiwifruit (*36*) and in cold-acclimated spinach plants (*16*), respectively. Low temperatures failed to induce significant increases in SPS and SS activities in the flavedo of nonconditioned fruit of the more chilling-susceptible cultivar (Navelate). Similar behavior was observed in fruit of the chilling-sensitive cultivar Fortune, whereas a decline in the activities of both enzymes occurred at the nonchilling control temperature (12 °C) (data not shown). Low temperature did not induce an increase in SS, but the activity of SPS rose in the chilling-tolerant ABA-deficient mutant (Pinalate) (**Figure 5**). Furthermore, the activity of both sucrose synthetic enzymes at 2 °C was higher in Pinalate fruit. Interestingly, sugars have been involved in signaling temperature changes in the environment for plants and may thus trigger metabolic changes such as an increase in ABA, resulting in the expression of proteins that are more functional at low temperatures (*40*). These higher activities did not counteract the decline in sucrose in nonconditioned fruit, which is consumed for respiration in detached citrus fruit after prolonged cold storage (*11*) and, therefore, the involvement of sucrose in the mechanism of cold adaptation of nonconditioned citrus fruit to chilling deserves further attention.

Our results also showed that the heat-induced sucrose accumulation occurring in the flavedo of both cultivars was accompanied by the activation of SPS and SS and that the increase in the activity of both enzymes was higher in Navelate than in Pinalate fruit. This trend is similar to that observed in potato leaves by Lafta and Lorenzen, who reported that the increase in SPS activity was associated with an increase in foliar sucrose in source leaves kept at high temperatures (*23*). SPS is a major control point for the partitioning of photosynthate between sucrose and starch in leaves (*41*), whereas in many carbohydrate sinks, sugars may be degraded and converted into starch. A comparison of the changes occurring in the starch and sucrose contents induced during heat treatment in Navelate and Pinalate fruit detached from the tree (**Figure 5**) and the fact that the starch levels in the flavedo of both cultivars were much less abundant than those of sucrose (**Figure 4**) suggest that the heat-induced sucrose did not arise from starch breakdown and, therefore, that alternative carbon sources were involved in the process.

Simple sugars and long-chain carbohydrate polymers have been reported to play a role in cold acclimation and also in

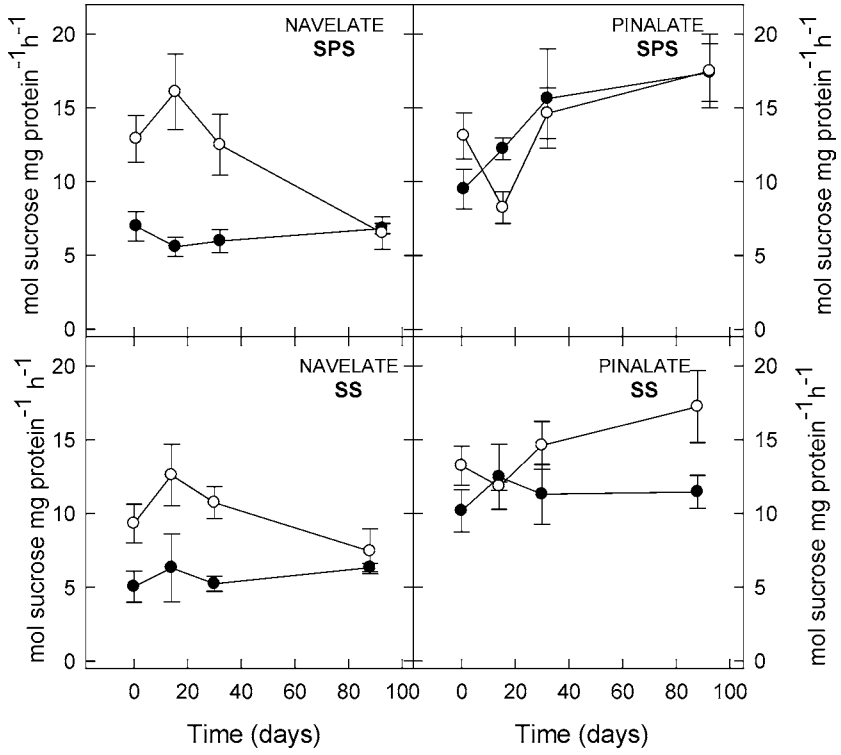

Figure 5. Extractable activities of SPS (upper panels) and SS (lower panels) in the flavedo of Navelate and Pinalate fruit stored immediately after harvest (nonconditioned fruit) or after 3 days of conditioning at 37 °C and 90–95% RH (conditioned fruit), with subsequent chilled (2 °C) storage. Oranges were exposed to the following treatments: (1) nonconditioned fruit stored at 2 °C (●); (2) conditioned fruit stored at 2 °C (○). Values are the means ± SEM of three replicate samples containing 10 fruit.

heat tolerance (*42*). Whether the heat-induced increase in sucrose found here and in the SPS and SS activities was a defense response of the citrus fruit against heating deserves further consideration. As the effect of the heat treatment in preventing sucrose decline in oranges during their storage at 2 °C may be associated with the changes induced in the SPS and SS activities, we cannot rule out the possibility that these enzymes might play a role in heat-induced chilling tolerance in Navelate fruit by increasing and maintaining higher sucrose levels.

The involvement of ABA in changes in carbohydrate levels and in SS and SPS activities has been reported in plants, although controversial results have been found (*18*, *19*, *43*). Cold stress favored SPS in the ABA-deficient cultivar but not in Navelate fruit and did not alter SS in nonconditioned fruit of any cultivar. However, the heat treatment (37 °C/3 days) induced a higher increase in SPS and SS activities in Navelate fruit, which may indicate the participation of ABA favoring the induction of both enzymes in response to high temperature in citrus fruit. Increases in SPS mRNA levels have also been related to the hormone ethylene (*36*), and heat conditioning may favor ethylene production in citrus fruit (*44*). However, no differences in the heat-induced changes in ethylene production were found between Pinalate and Navelate fruit in the present work (data not shown).

In conclusion, the ABA-deficient yellow Pinalate orange showed higher sucrose and starch levels and higher SPS and SS activities than its parental Navelate fruit, but was more susceptible to the development of RS. Heating the fruit favored the activation of these sucrose synthetic enzymes in concordance with the accumulation of sucrose but did not prevent RS. In addition, little difference was found in the reducing sugar contents in both cultivars or in response to heating. Therefore, carbohydrates appear not to play a role in protecting citrus fruit against this nonchilling physiological disorder. Conversely, because Pinalate fruit did not develop chilling symptoms and CI was prevented by heat conditioning in Navelate fruit, we cannot rule out the contribution of carbohydrate metabolism in the susceptibility of orange fruit to chilling.

ABBREVIATIONS USED

ABA, abscisic acid; CI, chilling injury; RS, rind staining; SPS, sucrose phosphatase synthase; SS, sucrose synthase.

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