The Development and Control of Husk Scald on ‘Wonderful’ Pomegranate Fruit during Storage

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Abstract. A superficial brownish discolouration (scald) develops on the husk of ‘Wonderful’ pomegranate (Punica granatum L.) fruit during storage. The severity of this disorder could be diminished by delaying the harvest time and by reducing storage temperature, but these 2 measures were insufficiently effective for storage periods exceeding 6 weeks, and at temperatures of 6°C or lower, chilling injury also occurred. Scald incidence was correlated with the amount of o-dihydroxyphenols extractable from the husk, and was significantly controlled by measures that inhibited their oxidation by polyphenol oxidase. Such postharvest measures included dipping the fruit in boiling water for 2 minutes and in anti-oxidant solutions, in bisdithiocarbamate-containing maneb for 30 seconds, or storing the fruit in a low-O₂ atmosphere. The most effective control of husk scald was obtained by storing late-harvested fruit in 2% O₂ at 2°C. However, this treatment resulted in accumulation of ethanol, which caused off-flavor development. When the fruit were transferred to air at 20°C, ethanol and off-flavors dissipated.

The ‘Wonderful’ pomegranate is harvested in Israel from mid-September to mid-October. A large percentage of the fruit is stored to enable its export to Europe for the Christmas season. Very little is known about the optimal storage conditions for this fruit, and various blemishes on the fruit husk have been observed in different storage seasons and under different commercial storage conditions. One of the most prevalent types of disorder is a brown, superficial discoloration of the husk, somewhat similar to the superficial scald of apples. At advanced stages of development, the scalded areas become mold-infected, and some softening of the fruit can occur as a consequence. However, in general, no internal changes have been observed in the appearance of the arils or of the white segments, as may result from chilling (8).

Browning of plant tissues—and of fruit tissues in particular—has generally been attributed to oxidative processes (7, 14, 16, 17). Oxidation may be either enzymatic (7, 14) or nonenzymatic (16, 17), and the remedy will, to some extent, depend on the type and nature of oxidation taking place (6, 7, 10, 14, 17). The most common type of nonenzymatic oxidation is a result of reactions between reducing sugars and amines—the Maillard reaction (16)—that, like other nonenzymatic oxidations, is enhanced by heat and humidity. Enzymatic oxidation of phenols is the most common cause of naturally occurring tissue browning, and the enzyme predominantly responsible is polyphenol oxidase (PPO) (3, 7, 14, 15, 20). Certain instances of tissue oxidation have been successfully prevented by application of antioxidants (6, 18).

The objectives of the experiments described here were to determine some of the factors that affect the development of husk scald on pomegranate fruits; to ascertain whether the brown discoloration was the result of enzymatic or nonenzymatic oxidation; and, on the basis of these findings, to examine some methods to prevent the development of husk scald on stored pomegranates.

Materials and Methods

‘Wonderful’ pomegranate fruit were harvested in 1981 and 1982 from 2 orchards grown under different climatic and edaphic conditions. Ten fruit were packed in cartons lined with perforated, 0.02-mm polyethylene film and stored on the day of harvest. The development of husk scald was monitored periodically, and a scald index was calculated as follows: percentage of fruit with 1–25% scalded surface + 2 × percentage of fruit with 26–50% scalded surface + 3 × percentage of fruit with 50% scalded surface.

The effect of storage temperature on scald development. Fruit harvested at the beginning of the harvest season (19 Sept.) were stored at 2°C, 6°C, or 10°C on the day of harvest and examined periodically until 23 Feb. The development of husk scald and other disorders was evaluated visually on 4 replicates (of 10 fruit) per treatment.

The effect of harvest time on scald development. Fruit were harvested and packed in 3 cartons (replicates) each containing 10 fruit, once a week for 5 weeks (from the end of September until the end of October) and stored at 10°C. The same fruit were examined on 4 occasions until the end of January and were then transferred to storage at 20°C for 1 week. At each harvest and at the end of the storage period, 5 replicates of 2 fruit were sampled for the following tests:

a) Polyphenol oxidase activity: 2 g of husk homogenized on ice with 20 ml 0.2 M cold phosphate buffer (pH 6.5) and 0.2 g Polyclar AT (BDH Chemicals, Poole, U.K.). The supernatant obtained after centrifuging at 5000 × g for 20 min served as the enzyme extract. The reaction mixture consisted of 2 ml extract and 1 ml 0.02 M 3,4-dihydroxyphenylalanine (dopa) at 25°C. The changing absorption at 475 nm was followed for 5 min (9). Protein was assayed in the extract by the method of Bradford (1).

b) o-dihydroxyphenol content: 2 g of husk was homogenized with 20 ml methanol and filtered. The filtrate was diluted and assayed according to the method of Mapson et al. (12). A standard curve was prepared with caffeic acid.

The effect of antioxidant treatments on scald development. ‘Wonderful’ pomegranates were dipped for 30 sec on the day after harvest in the following antioxidants: α-tocopherol (10⁻⁴ M), butylated hydroxy toluene (10⁻⁴ M), butylated hydroxy aniline (10⁻⁴ M), propylgallate (10⁻⁵ M), ethoxyquin (2000...
The development of husk scald on 'Wonderful' pomegranates stored at different temperatures.

Fig. 2. The effect of harvest date and storage duration on husk scald development on 'Wonderful' pomegranates at 10°C. Different letters represent significantly different values (P = 0.05), according to Duncan's multiple range test.

The effect of reduced oxygen in the storage atmosphere on the development of husk scald. The 1981 experiment was conducted in a continuous flow-through system. Air streams containing mixtures of N and 1%, 2%, 4%, or 21% O2 were passed through 15-liter glass jars containing 15 to 20 fruit each. These fruit were harvested at the beginning of the harvest season in each location. For each O2 treatment, 2 jars of fruit from each location were stored at 6°C and 10°C. The same fruit were examined once a month for scald development, and after 4 months they were transferred to air at 20°C for 1 week.

In 1982, the fruit were stored in a static atmosphere obtained inside sealed polyvinyl chloride (PVC) tents from which the O2 was removed by flushing with N at the beginning of the experiment and after each examination of the fruit. A buildup of CO2 above 0.5% in the tent was prevented by scrubbing with lime. The experiment consisted of 8 treatments: on each of 2 harvest dates fruit were stored at 2 levels of O2 (2% and 21%) and at 2 temperatures (2°C and 6°C). Six polyethylene-lined plastic boxes containing 20 fruit each were packed for each treatment. The development of scald was monitored on the same fruit throughout the storage period.

At the end of the storage period and after 1 week at 20°C, fruit were sampled by a taste panel of 9 to 11 persons, who were asked to define the fruit as tasty, edible, or inedible and to grade the presence of off-flavors from 0 (none) to 3 (severe).

The amount of ethanol that accumulated in the fruit was estimated by collecting 10 ml of juice from 2 fruit per replicate in a 50-ml flask. The flask was sealed immediately with a rubber septum and placed in a shaking bath at 30°C for 7 min. A 2-ml sample from the head space was then withdrawn and injected into a gas chromatograph equipped with a 20% carbowax 20-M column (1.85 m long with a 2-mm i.d.) and flame ionization detector. The carrier gas was N, injection temperature 110°C, oven temperature 100°C, and detector temperature 180°C. Standards of 100, 500, and 1000 μl-liter⁻¹ ethanol were prepared and assayed in the same manner as the juice samples.

Results

Husk scald began to appear at the beginning of November on 'Wonderful' pomegranates that had been harvested at the end of September and stored at 6°C or 10°C (Fig. 1). The severity of scald was higher at 10°C than at 6°C, and, as the rate of development was about the same at both temperatures, the symptoms probably began to appear earlier at 10°C than at 6°C. At 2°C the onset of the disorder was delayed until the end of December, but its development thereafter was rapid. In addition, chilling injury (in the form of necrotic pitting of the husk) occurred in November at this storage temperature.

The onset of husk scald could be delayed and its severity reduced by postponing the date of harvest (Fig. 2). However, at the end of January the incidence of scald was quite high, even in fruit harvested late in October. (Fruit harvesting could not be postponed further because the husk began to split and the incidence of decay in the orchard and in storage would have been increased considerably.)

Considering the possibility that the browning of the husk is the result of phenol oxidation, the amount of o-dihydroxyphenols extractable from the husk at harvest was assayed and compared with the incidence of scald that developed at 10°C (Fig. 3). The level of o-dihydroxyphenols decreased by 48% during the month of harvest and the correlation to storage scald was high and significant (r = 0.86, p = 0.001). There was no significant change in the amount of extractable o-dihydrophenols during storage at 10°C (data not shown).

Polyphenol oxidase activity, assessed in a crude phosphate-buffer extract (pH 6.5) from the husks, was shown to be completely inhibited by 10 μmol diethyldithiocarbamate (DETC) (Fig. 4) or by treatment for 5 min at 100°C. However, no change in the specific activity of PPO occurred during the harvest season. At the end of the storage period at 10°C (23 Jan.), PPO activity was about 20% of that at harvest, but again, no differences were measured between extracts from fruit of different harvests nor between fruit with or without husk scald (data not shown).
Fig. 3. The effect of harvest date on the amount of o-dihydroxyphenols extractable from the husk of ‘Wonderful’ pomegranates at harvest (A) and their correlation with the average incidence of husk scald that developed during 4 months of storage at 10°C (B).

Fig. 4. The effect of diethyl dithiocarbamate on polyphenol oxidase activity at 25°C. Incubation medium: 1.5 ml extract in 0.2 M phosphate buffer, pH 6.5; 1.0 ml 0.2 M DL-dopa; and 0.5 ml DETC at specified concentrations.

Fig. 5. The effect of hot water and bisdithiocarbamate dips on the development of husk scald on ‘Wonderful’ pomegranate stored at 10°C. Different letters represent significantly different values (P = 0.05) according to Duncan’s multiple range test. Pomegranates dipped for 2 min in boiling water or in the fungicide maneb (active ingredient bisdithiocarbamate, a chelate that also complexes copper), and stored at 10°C showed significantly reduced scald after 2 and 3 months of storage (Fig. 5). The hot-water dip completely inhibited scald development, indicating the probability that this peel disorder is the result of enzymatic oxidation. Antioxidants, applied (like maneb) as postharvest dips, were equally effective in delaying scald development (data not shown), but none gave commercially acceptable control of scald during 3 months of storage at 10°C.

In a continuous-flow system, scald development was inhibited at reduced O2 tensions and progressed at increasingly slower rates as the O2 level was lowered, being more effectively inhibited by reduced O2 levels at 6°C than at 10°C (Fig. 6). However, when the healthy or the least-scalded fruit was transferred to air at 20°C, there was an increase in scald incidence, especially in fruit that had been stored at 10°C. Ethanol accumulated markedly in fruit during storage when the O2 tension was below 2%, and 6 days at 20°C in air were insufficient to totally dissipate.

Fig. 6. The effect of O2 levels in a continuous flow system on husk scald development on ‘Wonderful’ pomegranates stored at 6° or 100°C, then transferred to air at 20°.

The effect of storage temperatures and O_2 level on the accumulation of ethanol in the juice of 'Wonderful' pomegranates during storage and its dissipation at 20°C in air.

The inhibitory effect of reduced O_2 in the storage atmosphere on the development of husk scald on pomegranates indicates that the disorder is the result of an oxidation process that occurs in harvested fruit. Furthermore, the total prevention of scald development on fruit that was immersed for 2 min in boiling water suggests that the oxidation is enzymatic in nature and could well be induced by PPO (13). The activity of PPO—as the most likely cause of oxidative browning (14)—did not change during the harvest season, even though the susceptibility of the fruit to scald development declined the longer the fruit remained on the tree. Moreover, with the development of scald during storage, PPO activity declined. On the other hand, although scald development could not be related directly to enzyme activity, a good correlation with the amount of its o-dihydroxyphenol substrate was shown. Similar correlations between tissue browning and the amount of phenolic substrate have been reported for apple (5), banana (19), potato (12), and avocado (4). In avocado, however, a correlation with PPO activity was also observed (9). The involvement of PPO in pomegranate scald was also suggested by the significant, albeit insufficient, control of scald achieved by treatment with maneb. This control could be attributed to the inhibition of PPO resulting from the chelation of the copper ion in the prosthetic group by bisdithiocarbamate, the active ingredient of maneb (3).

The effectiveness of reducing storage temperature and O_2 tension in controlling or delaying husk scald development also lends support to the hypothesis that the disorder is the result of enzymatic oxidation. At 4% O_2, the inhibition of scald was significant compared with the control, but it was insufficient and much less effective than at 2% O_2. The effect was increased by reducing O_2 from 2% to 4%, possibly because of the affinity of PPO for O_2. The Km for potato PPO was reported by Mapson and Burton (11) to be 1.5–6.0 x 10^{-4} M. If the calculations of Burton (2) for potato can be used as a rough guideline, the concentration of dissolved oxygen in the cells of fruit held in 4% O_2 at 6°C would be about 10% of this value. It would therefore be expected that, with a further reduction in the oxygen level, the PPO activity would be drastically curbed and scald controlled more effectively. The eventual appearance of scald, even at 1% O_2, indicates that the enzyme was not totally inactivated, but rather that its activity was reduced by the environment. Moreover, there is the possibility that the permeability of the outer skin and the inner membranes to O_2 diffusion into the cell increases with the senescence of the fruit (2).

The reduction in O_2 also inhibited fruit respiration, and although the affinity of cytochrome oxidase for O_2 is high (K_m = 0.5 μM), the accumulation of ethanol at 2% O_2 (about 0.6 mM) shows that the activity of the anaerobic glycolytic pathway must have increased, and that of cytochrome oxidase diminished. Ethanol accumulation increased as the temperature rose and/or the O_2 level declined, both of which limited the O_2
supply ethanol relative to the respiration rate. However, the dissipation of ethanol upon removal of the fruit to air at 20°C provided a practical solution to the prolonged storage of ‘Wonderful’ pomegranates. The objective of determining an O₂ level in storage that would control the development of husk scald without incurring overproduction of ethanol due to anaerobic respiration seems to have been achieved for storage periods of 3 to 4 months. It is possible that improved fruit quality and extended storage life might be obtained, and with this goal in mind, O₂ concentrations between 2% and 4% at temperatures between 2° and 6°, should be examined.

**Literature Cited**