Forced Hot Air Treatment of Stone Fruit to Inhibit the Development of Mealiness

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Abstract
Treatment with high temperature forced air (HTFA) was tested as a potential alternative to the industry practice of preconditioning stone fruit at 20°C for 2 days to slow the development of mealiness during cold storage. Preconditioned (PC) fruit and non-PC fruit were treated with HTFA (final temperature = 46°C) for 1 to 4 hours and placed into cold storage for 2 to 4 weeks at 1°C. Juiciness of the fruit was quantified after ripening by measuring the percentage of free juice (FJ) present. Fruit stored for 2 weeks were juicy regardless of treatment, although 3 to 4 hours of HTFA treatment was needed for the percentage FJ in non-PC fruit to be similar to that present in PC fruit. After 3 weeks of storage non-PC fruit that had not been heated had a FJ value of 27% as compared to 50% for non-heated PC fruit and had become mealy. Heating slowed the loss of FJ and maintained juiciness, although 3 hours of heating or more was required to have an effect and the FJ value for the non-PC fruit heated for 4 hours was still 7% less that that from non-heated PC fruit. Both PC and non-PC fruit had become mealy by the end of 4 weeks of storage and had low FJ values. Exo-polygalacturonase, endo-polygalacturonase and pectinmethylesterase activities in the tissues were affected by PC and heating but did not change in a manner that indicated that these enzymes could be responsible for the differences in FJ. Preconditioning increased the abundance of the protein expansin in non-heated samples but HTFA treatment strongly inhibited its accumulation. High temperature forced air can effectively reduce the incidence of mealiness but further research is needed to determine if the technique could be useful commercially.

INTRODUCTION
Mealiness is a severe storage disorder of stone fruit that causes the flesh to develop a dry texture. Although primarily associated with cold storage, high temperature treatment can also influence the development of mealiness in stone fruit (Obenland and Carroll, 2000). The authors found that fruit heated using high temperature forced air (HTFA) to a seed-surface temperature of 47.2°C had a greater tendency to become mealy than did non-treated fruit. More recently, heat treatments have been conducted with peaches and nectarines using a HTFA protocol designed to disinfest stone fruit of Oriental fruit moth (L. Neven, personal communication). This treatment protocol, which differed from the previously used treatment in heating rate, overall treatment time and final seed surface temperature (46°C), did not cause a strong enhancement in the development of mealiness (Obenland, unpublished data) and in many instances inhibited mealiness. The same work has shown that in some peach and nectarine varieties, such as the peach ‘Elegant Lady’, heat can strongly promote as well as inhibit the development of mealiness, depending on the duration of the HTFA treatment. One objective of this work was to compare HTFA with pre-conditioning, which is an industry practice to store fruit at 20°C for 48 h prior to cold storage to reduce the incidence of mealiness, to determine the relative effectiveness of the two methods in inhibiting the development of mealiness. A further objective was to use the differential effect of heat on mealiness in the variety
‘Elegant Lady’ to enhance understanding of the biochemical basis of the disorder by examining the activities of key cell wall lytic enzymes and expansin protein abundance following different HTFA treatments.

MATERIALS AND METHODS

Peaches (cv. ‘Elegant Lady’) of commercial maturity and classified as size 56 were obtained from a local packing house and transported back to the laboratory. A portion of the fruit that were to be pre-conditioned (PC) were placed at 20°C while the rest was put into cold storage at 1°C. Pre-conditioning was conducted at 20°C for a period of 36 hours after which the fruit were transferred to 23°C for 12 hours. Non-preconditioned fruit were removed from cold storage and placed at 23°C for 12 hours immediately prior to heat treatment. Each of the 4 replications received the same storage protocols specific for PC or non-PC fruit prior to treatment.

Heat treatments were conducted over a total of 4 days, performing one replication each of PC or non-PC fruit per day, using a treatment chamber designed by Techni-Systems (Chelan, WA, USA) and very similar to that described in Neven and Mitcham (1996). Temperature probes were used to monitor fruit surface and seed surface temperatures during the runs. Thirty fruit were treated per replication. Treatments were initiated using a chamber temperature of 23°C followed by a 12°C/hour upward ramp up to a final chamber temperature of 46°C, where the temperature was held to complete a total treatment time of 4 hours. Controls were held untreated in the treatment room for the same amount of time. Controlled atmospheres, although a normal part of the heat treatment protocol for insect disinfestations, were not used in this test. Dewpoint was maintained 2°C below the temperature of the coolest probed fruit. Fan speed was set to maintain airflow through the fruit at 2 m/s. Following the completion of treatment fruit were removed from the treatment chamber and placed into storage at 1°C.

After the completion of 2, 3 or 4 weeks of cold storage fruit were moved from cold storage to a room set at 23°C to allow the fruit to ripen. Penetrometer readings (University of California Firmness Tester) were taken periodically to determine when fruit had reached a firmness of 13.4 N or less at which point the fruit were considered ripe. Five fruit from each treatment lot were randomly selected used to estimate the percentage of free juice in the fruit using the method of Crisosto and Labavich (2002). Longitudinal slices were also removed from the sampled fruit, frozen in liquid nitrogen and placed into storage at -80°C for enzymatic assays and Western blot analyses.

The enzyme extraction procedure was adapted from that described in Buescher and Furmanski (1978). Following the initial extraction the extract was desalted by passage through a Sephadex G-25 desalting column. Enzyme assays for both exo- and endo-acting polygalacturonase (PG) activity was as described in Artés et al. (1996) and is further detailed in Obenland and Carroll (2000). Quantification of the PG activities was done by determination of reducing groups (Gross, 1982). The same extract was used to assay PG and also to determine pectinmethylesterase activity with the gel diffusion assay of Downie et al. (1998). Four replications were done for each treatment with separate fruit samples being extracted for each replication.

Protein extractions similar to Brumell and Harpster (1999) were performed using the same tissue samples as were used for the enzymatic assays. The resulting extracts were subjected to SDS-PAGE and the proteins transferred to nitrocellulose. Protocols for protein separation, protein blotting and probing of the blots with expansin LeExp1 antibodies is given in Obenland et al. (2003).

Analysis of variance for the FJ data was conducted using storage time, heating duration, conditioning, and the interactions as fixed effects. The random effects were replications and the two- and three-way interactions of the fixed effects with replications. Means and 95% confidence intervals were calculated and used to determine the statistical significance of mean comparisons. The enzyme data, being only taken from tissue stored for 3 weeks, were analyzed in the same manner but without storage duration as a fixed effect.
RESULTS AND DISCUSSION

Free Juice

Percent free juice measurements for pre-conditioned (PC) and non-PC fruit following 2, 3 and 4 weeks of cold storage is presented in Fig. 1. After two weeks of storage PC fruit remained very juicy as indicated by the high FJ readings (Fig. 1A). The FJ levels were very similar to those obtained in fruit that had not undergone cold storage (data not shown) and were not affected by heat treatment. Fruit that had not been pre-conditioned, while still juicy, had lower FJ, indicating that the disorder of mealiness had begun to manifest itself. Fruit treated with 3 or 4 hours of heat treatment, however, were less mealy than control, 1 hour- and 2 hour-treated fruit, with FJ values approaching those of the PC fruit. Following three weeks of storage the control non-PC fruit had dropped to 25.9% FJ and were very mealy as compared to 49.6% FJ for PC fruit, which were still juicy (Fig. 1B). This inhibitory effect of pre-conditioning on the development of mealiness has been previously reported (Crisosto et al., 2004) and is the primary reason that pre-conditioning prior to cold storage is practiced commercially in California. At this storage time heating initially enhanced mealiness in the PC fruit as the percent juice in the 1-hour treatment dropped slightly from the control value to 44.7% and then to 24.9% for the PC fruit treated for 2 hours. Similarly, in non-PC fruit, heat promoted the development of mealiness after 2 hours of heating but inhibited mealiness in the 1-hour treatment. In both PC and non-PC fruit further heating (3 and 4 hour treatments) progressively increased the percent FJ until the levels were equal to or greater than the control values. After 4 weeks of storage non-PC fruit from all of the treatments were mealy with FJ values ranging from 20.0 to 32.2%, and none of the treatments were significantly different (P≤0.05) from each other (Fig. 1C). Control, 2 and 3 hour treatments of the PC fruit were also mealy with FJ values not different statistically (P≤0.05) from non-PC fruit. Preconditioned fruit that had been heated for 4 hours were juicier than non-heated fruit.

Enzymatic Activity

Exopolygalacturonase (Exo-PG), endopolygalacturonase (Endo-PG) and pectinmethylesterase (PME) activities following treatment and 3 weeks of storage were determined to investigate whether changes in these key cell wall lytic enzymes could be the basis for the observed differences in FJ (Fig. 2). In the control (non-heated) fruit activities of all three enzymes were significantly higher (P≤0.05) in PC fruit as compared to non-PC fruit. Previous work (Zhou et al., 2000) had suggested that the beneficial effect of pre-conditioning was due to maintenance of a high PG/PME activity ratio during and following cold storage. Although PG activity was definitely higher in non-heated PC fruit in this study, PME activity was also enhanced, actually leading to a lower PG/PME ratio in the PC fruit. Heating for 1 hour had little or no effect on the enzyme activities of PC fruit. Previous work (Zhou et al., 2000) had suggested that the beneficial effect of pre-conditioning was due to maintenance of a high PG/PME activity ratio during and following cold storage. Although PG activity was definitely higher in non-heated PC fruit in this study, PME activity was also enhanced, actually leading to a lower PG/PME ratio in the PC fruit. Heating for 1 hour had little or no effect on the enzyme activities of PC fruit. Further heating caused a slow but progressive decline in exo-PG activity for both PC and non-PC fruit. Endo-PG activity declined by 45 and 43%, for PC and non-PC fruit, respectively, in response to increasing heat duration from 1 to 2 hours. From 2 hours of heating onward endo-PG activity leveled off and there were no statistically significant changes in activity due to additional heating. Similarly, PME activity declined from 1 to 2 hours of heating in both PC and non-PC fruit and did not change substantially from that point onward. Comparison of these enzymatic changes in the HTFA-treated samples with the changes in FJ of the same samples indicated that there was no clear trend for higher FJ samples to have correspondingly higher PG/PME enzyme activity ratios.

Expansin Protein

A cross-reacting 27-kD protein, matching the predicted molecular mass of peach expansin (Hayama et al., 2000), was present in tissue from both PC and non-PC fruit that
had not been heated but was not detected in either of the HTFA-treated samples (Fig. 3). Comparison of the results from the two HTFA-treated samples indicated that that a greater abundance of expansin protein was present in the juicy PC tissue in contrast to the mealy non-PC tissue. This supports previous work that indicated a potential link between the development of mealiness and a loss of expansin protein during cold storage (Obenland et al., 2003). In this case, however, none of the two HTFA-treated samples contained detectable amounts of expansin, even though heating for 4 hours enhanced the FJ content over that present in the non-heated tissue.

CONCLUSIONS
As seen in previous studies (Crisosto and Labavich, 2002; Nanos and Mitchell, 1991) this research also showed that pre-conditioning of fruit prior to the initiation of cold storage helped maintain the juiciness of the fruit during and following cold storage. This procedure, however, requires that the fruit be pre-ripened for 2 days which is a rather lengthy procedure and more importantly may predispose it to other storage problems such as enhanced decay rates. High temperature forced air treatment is a rapid process, does not enhance ripening of the fruit, and may offer an alternative treatment for slowing the development of mealiness. This study indicated that HTFA treatment given for a duration of 3 hours or more was able to almost completely substitute for PC when the fruit were stored for 2 weeks. Following 3 weeks of storage, HTFA treatment also helped maintain the juiciness of the fruit but the FJ in the PC fruit was still slightly greater than that of the HTFA-treated fruit. The individual inhibitory effect of both PC and HTFA treatment on the development of mealiness was lost after 4 weeks of storage as the control PC fruit and all of the non-PC fruit became mealy. In this case treatments using both HTFA treatment and PC were the most successful. Although more work needs to be done to ascertain the effects of heat treatment on additional varieties differing in their susceptibilities to mealiness and to further define the effect of heat on other attributes of fruit quality it appears that HTFA treatment holds promise as a potential substitute for PC.

Activity changes in exo-PG, endo-PG and PME did not readily explain how heating or PC affected the development of mealiness in this study. Although a high PG/PME activity ratio has been suggested to be key to maintaining the juiciness of fruit during and following cold storage (Zhou et al., 2000) it is possible that HTFA treatment may alter FJ by an alternative mechanism. It is also possible that multiple factors in addition to PG and PME are involved in determining the FJ present in stone fruit in both HTFA-treated and cold-stored fruit. One potential factor that has been associated with the development of peach mealiness in a previous study (Obenland et al., 2003), is the protein expansin. Although a similar positive relationship was found between expansin abundance and FJ in non-heated fruit, the same relationship did not appear to exist in HTFA-treated fruit where expansin accumulation during ripening was strongly inhibited.

Literature Cited


Fig. 1. Free juice (FJ) percentages following HTFA treatment and storage for 2, 3 or 4 weeks at 1°C and subsequent ripening for 3d at 23°C. Each data point represents the mean (±SE) of 4 replications with 5 fruits per replication.
Fig. 2. Activities of exo- and endo-polygalacturonase and pectinmethylesterase following HTFA treatment and storage for 3 wks at 1°C and ripening for 3d at 23°C. Each data point represents the mean (±SE) of 4 separate assays using different tissue samples.
Fig. 3. Immunodetection of a 27-kDa expansin protein present in PC and non-PC fruit subjected to different durations of HTFA heating and stored for 3 weeks at 1ºC.