

Mealiness and Pectolytic Activity in Peaches and Nectarines in Response to Heat Treatment and Cold Storage

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ABSTRACT. ‘Elegant Lady’, ‘O’Henry’ and ‘September Sun’ peaches [(*Prunus persica* (L.) Batsch (Peach Group))] and ‘Summer Bright’ and ‘Summer Grand’ nectarines [(*Prunus persica* (L.) Batsch f. *nucipersica* (Nectarine Group))] heated to a seed surface temperature of 47.2 °C over a period of 4 hours developed mealy flesh sooner and to a much greater extent than nonheated fruit following cold storage at 5 °C for 1 to 3 weeks. Exo- and endopolygalacturonase activities were reduced following 3 to 4 hours of heating and may have been responsible for the increased mealiness. Mealiness often developed in defined regions rather than throughout the entire fruit. Comparison of juicy and mealy regions within individual fruit revealed that mealy regions contained 65% and 86% less exo- and endopolygalacturonase activity, respectively, than juicy regions, whereas pectinmethylesterase activity was unchanged. Extractable protein was reduced by >50% in the mealy regions of the fruit. Intermittent warming periods of 24 hours at 20 °C at weekly intervals during storage at 5 °C were less effective in reducing mealiness in heat-treated than in control fruit. It is important that future work with heat treatments and stone fruit closely monitor potential effects on this disorder to avoid loss of market quality following treatment.

Quarantine requirements of several importing countries require a pest disinfection treatment of stone fruit. This quarantine treatment has been accomplished traditionally by chemical fumigation using methyl bromide. The use of chemical fumigants, however, is becoming increasingly problematic due to legislative pressures to ban or restrict their use. As a result, there is great interest in developing alternative means of insect disinfection that are nonchemical.

Heat is a very efficacious treatment that has been tested on many commodities (Couey, 1989). Research by our group (Obenland et al., 1999) has indicated the potential feasibility of using high temperature forced air (HTFA) as a quarantine treatment for nectarines [*Prunus persica* f. *nucipersica* (Nectarine Group)] by demonstrating that a treatment sufficient to kill Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) did not alter the market quality of the nine cultivars tested. The most noticeable effect on these nectarines was delayed ripening, similar to that shown for other commodities during storage at temperatures ranging from 30 to 40 °C (Eaks, 1978; Ogura et al., 1975a) or following short-term heat treatments before ripening (Klein and Lurie, 1990; Porritt and Lidster, 1978).

Internal breakdown is a serious physiological disorder of stone fruit that causes the flesh to brown and develop a mealy (also referred to as woolly) texture. The disorder is most apparent following storage temperatures of 2.2 to 7.7 °C, with symptoms being expressed during ripening (Lill et al., 1989). Delaying implementation of cold storage following harvest (Von Mollendorf et al., 1992) and periods of intermittent warming during storage (Buescher and Furmanski, 1978; Dawson et al., 1995; Fernández-Trujillo et al., 1998) can reduce the amount of internal breakdown. It is believed that the mealiness characteristic of internal breakdown is associated with a chilling-induced abnormality in the breakdown of pectin that leads to an accumulation of in-

soluble, low methoxy pectic substances that have relatively high molecular weights and the capability of forming gels (Ben-Arie and Lavee, 1971). Subsequent loss of free moisture due to gel formation results in dry, mealy fruit.

Alterations in the activities of polygalacturonase (PG, EC 3.2.1.15) and pectinmethylesterase (PME, EC 3.1.1.11), two important ripening-related enzymes, have been associated with impaired pectin solubilization and the development of mealiness. While PG is inhibited by low temperatures, the effect of cold on PME is variable (Ben-Arie and Sonogo, 1980; Buescher and Furmanski, 1978; Von Mollendorf and De Villiers, 1988). The change in the relative activities of the two enzymes is thought to lead to formation of gel-forming pectic compounds and a tendency for the fruit to become mealy.

Solubilization of pectin is a fundamental and important aspect of fruit ripening (Fischer and Bennett, 1991). The purpose of this study was to ascertain whether impairment of ripening that we have observed to occur following HTFA treatment affects the occurrence and expression of mealiness in stone fruit. In addition, the activities of PG and PME were determined to evaluate the potential role of these enzymes in the development of mealiness in heat-treated stone fruit.

Materials and Methods

HIGH-TEMPERATURE FORCED-AIR TREATMENT—1998. Commercially packed ‘Summer Bright’ and ‘Summer Grand’ nectarines and ‘Elegant Lady’ peaches [*Prunus persica* (Peach Group)] were obtained 20 July from a packing house in the vicinity of Fresno, Calif. All of the fruit had been harvested at a maturity stage (California well matured) normally used for commercial shipping. The three cultivars chosen for the experiment and their average individual fruit weights were ‘Summer Grand’ (172.5 g), ‘Summer Bright’ (171.0 g) and ‘Elegant Lady’ (164.1 g). ‘Elegant Lady’ peaches and ‘Summer Bright’ nectarines are susceptible to the development of mealiness, while ‘Summer Grand’ is a relatively nonsusceptible nectarine cultivar (Crisosto et al., 1999).

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Heat treatments were performed at the University of California Lindcove Research and Extension Center, Lindcove, Calif., using a forced-air heat treatment chamber (Aquanomics Intl., Honolulu, Hawaii). The chamber and its operation were as described previously (Obenland et al., 1999). Seed surface temperatures were monitored using a thermocouple input module (Daqbook 112/DBK 52; Iotech, Inc., Cleveland, Ohio) coupled to laptop computer and using thermocouples (Type T; Gaffney Engineering, Gainesville, Fla.). Temperature was increased rapidly within the chamber to 35 °C, and then linearly ramped up to 48.5 °C over a period of 200 min. This chamber temperature was maintained until termination of the experiment when the total treatment time reached 4 h. Relative humidity (RH) during the run was increased in a stepwise fashion, beginning at 60% RH for the first hour, increasing to 80% RH for the second hour and finally increasing to 90% RH for the rest of the run. During a run a portion of the fruit were removed after 2, 3, and 4 h of heating to observe the effect of increasing duration of heating upon mealiness and PG activity. At the completion of the run, the fruit were placed for either 1 or 2 weeks into cold storage at 5 °C, a temperature known to be permissive to the development of mealiness (Mitchell et al., 1974). RH during storage was 65%. Mealiness was also found to be enhanced by heat treatment in fruit stored at 0 °C (data not presented), but 5 °C was chosen for further experiments in order to enhance and be better able to study treatment effects. Following cold storage, the fruit were placed at 22 °C and 50% RH for 3 d to ripen and then evaluated for mealiness. Tissue samples were taken randomly from the equatorial region of each fruit in the form of plugs 1 cm in diameter, extending from the skin to the pit, and frozen by placing the samples in a freezer at -20 °C to permit later biochemical analyses. Each treatment (heating + storage time combination) was performed with three replicates of 20 fruit for each treatment, each replication being a separate heat treatment run. All three replications were from the same lot of fruit.

HIGH-TEMPERATURE FORCED-AIR TREATMENT—1999. In the second year of the study the peach cultivars used were ‘O’Henry’ and ‘September Sun’ with a fruit size of \approx 170 g per fruit. Both peach cultivars were classified in previous work as highly susceptible to the development of mealiness (Crisosto et al., 1999). All fruit were commercially packed and obtained from a packing house near Fresno, Calif., and were of a maturity normally harvested for shipping.

Heat treatments were performed at the Agricultural Research Service lab in Fresno using a forced-air heat treatment chamber constructed by Techni-Systems (Chelan, Wash.). The specifics of the chamber have been described previously (Neven and Mitcham, 1996). The temperature protocol was the same as for the 1998 season. Measured fruit seed surface temperatures were very similar between runs made with the Aquanomics chamber used in the 1998 season and that constructed by Techni-Systems. Dewpoint temperature was maintained at 2 °C below the lowest probed fruit surface temperature and fan speed at 2.5 m·s⁻¹. Internal flesh temperatures were monitored at the seed surface using the Daqbook system as in the initial year of the study. Following the heat treatment run, the fruit were stored at 5 °C for 1, 2 and 3 weeks at 65% RH to allow the development of mealiness. To test the effect of intermittent warming on development of mealiness, portions of the fruit were removed after 1 and 2 weeks storage and allowed to stand at 20 °C and 50% RH for 24 h, whereupon the fruit were again moved into storage at 5 °C and 65% RH. As in 1998, the fruit were ripened for 3 d at 22 °C and 50% RH before any evaluation. The peach cultivar used for the intermittent warming

experiment was ‘September Sun’. Twenty fruit were used per treatment and each treatment was replicated three to four times by utilizing separate heat treatment runs.

Since mealiness often did not occur throughout an individual fruit, but in defined sectors or regions, samples of mealy and juicy tissue were excised from seven ‘O’Henry’ fruit that prominently displayed this characteristic and frozen at -80 °C for later analysis of PG and PME activity and protein content. These fruit were assayed individually to allow a direct comparison of the two tissue types (juicy/mealy) within individual fruit.

EVALUATION OF FRUIT FOR MEALINESS. All fruit were evaluated by utilizing a University of California Firmness Tester with a 7.9 mm diameter plunger and were found to be at a similar degree of firmness (\approx 5 N, data not presented). Fruit mealiness was estimated visually by removing a large slice from each fruit, squeezing the slice, and observing the ease with which juice could be expressed from the flesh. Browning and a grainy appearance of the flesh were also characteristics often associated with mealiness. Fruit displaying any degree of dryness were classified as mealy. Fifteen to 20 fruit were classified in this manner in each treatment for each of the three to four replications.

POLYGALACTURONASE ASSAY. The extraction procedure was adapted from that of Buescher and Furmanski (1978) and was initiated by homogenizing frozen tissue (5 g) with a mortar and pestle in 2.5 mL of grinding solution containing 0.05 M Na-acetate (pH 5.5), 0.5 M NaCl, and 1% PVPP. The extract was then passed through cheesecloth and centrifuged at 15,000 g_n for 15 min. The supernatant was assayed for protein using the method of Bradford (1976). Before assay the supernatant was desalted by passage through a desalting column containing Sephadex G-25 (3 mL bed volume) and equilibrated with 0.05 M Na-acetate (pH 4.0, endo-PG activity; pH 5.5 exo-PG activity). The enzyme assay for both exo- and endo-acting activity was performed as reported by Artés et al. (1996). The reaction mixture contained 50 mL 20 mM Na-acetate (pH 5.5), 12 mL 1% chloramphenicol, 12 mL CaCl₂ and 125 mL enzyme extract for the exo-PG assay and 50 mL Na-acetate (pH 4.0), 12 mL 1% chloramphenicol and 125 mL enzyme extract for the endo-PG assay. The reaction was initiated by addition of 125 mL 0.5% polygalacturonic acid and incubated at 30 °C with a reaction time of 24 h. The reaction was terminated by addition of 500 mL 400 mM borate buffer (pH 9.0) to the entire reaction mixture. Reducing groups were determined by the method of Gross (1982). Following addition of 100 mL 2-cyanoacetamide to the reaction mixture, the samples were placed into a boiling water bath for 10 min, cooled and then the absorbance was read at 276 nm. Polygalacturonic acid (Sigma Chem. Co., St. Louis, Mo.) was used as a standard and activity expressed in katal (mol reducing groups/s). Three separate tissue plugs from an individual fruit were homogenized together for each assay, and three fruit were assayed within each replication. Only fruit stored for 1 or 2 weeks were assayed due to there being relatively little difference in the incidence of mealiness beyond 2 weeks. For comparison of juicy and mealy regions within individual fruit, samples were taken from each region and three separate extractions made and assayed for each sample. Seven separate fruit were assayed in this manner and were used as replications.

PECTINMETHYLESTERASE (PME) ASSAY. An enzyme extract was prepared by homogenizing 0.5 g of peach tissue in 0.5 mL of low salt extraction buffer (10 mM sodium phosphate, 0.5 % PVPP, pH 7.0) and centrifuging in a microcentrifuge at 15,000 g_n for 15 min. The supernatant was discarded and the pellet resuspended in 1 mL high salt extraction buffer (50 mM sodium phosphate, 1.5 M NaCl,

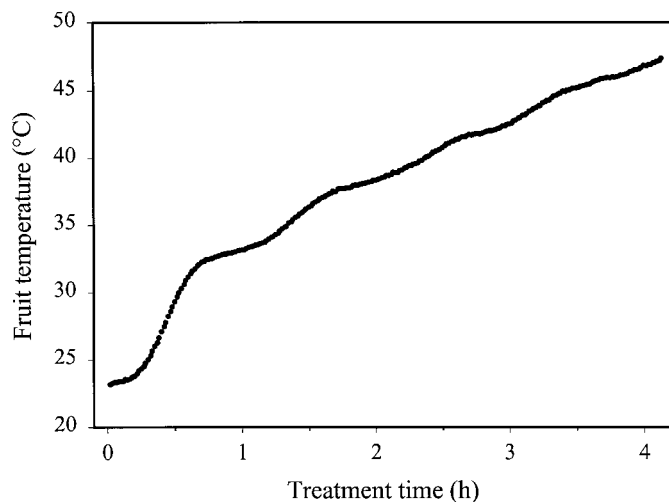


Fig. 1. Fruit internal temperatures as measured at the seed surface from a typical HTFA treatment run in the 1998 season. Temperatures were recorded once per minute. Each point represents a mean of the temperatures from 10 fruit.

15 mM EDTA, pH 7.0) and shaken for a period of 1 h. The extract was then centrifuged at 15,000 g_n for 15 min and the supernatant desalted by passage through a column containing Sephadex G-25 (3 mL bed volume) that had been equilibrated with 50 mM sodium phosphate buffer. The desalted extract was used for PME activity measurement and protein determination using the method of Bradford (1976). The assay was performed utilizing a gel diffusion assay by (Downie et al., 1998). Ten microliters of extract was loaded in each well. Activity, expressed in katals, was calculated by comparison to a standard curve made using orange peel PME as described by the authors. The extraction and assay was replicated seven times using seven separate fruit in which the individual fruit had been divided into juicy and mealy portions.

DATA ANALYSIS. Before statistical analysis, an arcsine square root transformation was used on the proportions of mealy and juicy fruit to stabilize variances. Mealiness data were analyzed by the least squares model (PROC GLM, SAS Institute, Inc., Cary, N.C.) with the main effects being storage time, cultivar, and heating time. Allowance was made for those groups that had all mealy or all juicy fruit by subtracting the error degrees of freedom for these groups from the main error degrees of freedom. None of the interactions between storage time, cultivar, and heating time were significant so inferences were directed to the main effects and orthogonal contrasts made for storage time and heating time. Mean separations were made within storage times for the intermittent warming data using Duncan's multiple range test.

Polygalacturonase data were analyzed using PROC GLM after transforming the exo-PG data by natural logs. F tests were conducted for storage time, cultivar, heating time, and interactions as well as orthogonal contrasts for treatment.

Comparison of the enzymatic activities and protein content of juicy and mealy tissue sectors (Table 1) was performed by pairing juicy and mealy data from the same fruit and running a paired *t* test.

Results

During the duration of the HTFA run, all of the probed seed surface temperatures exceeded 47.2 °C for at least 2 min (Fig. 1). This seed surface temperature was used as an endpoint to the heat

treatment as it fulfilled the criteria previously set for completion of a quarantine treatment for Mediterranean fruit fly (Obenland et al., 1999; U.S. Dept. of Agriculture, 1998).

High-temperature forced-air treatment acted to accelerate development of mealiness in all three of the cultivars tested (Fig. 2). Following the first week of storage mealiness was detected only in HTFA-treated fruit, with ≥ 3 h heat required in 'Summer Grand' and 'Summer Bright' nectarines, and 2 h in 'Elegant Lady' peaches. The percentage of mealy fruit increased greatly after 2 weeks of storage but changed much less from week 2 to week 3. After 2 weeks, the heat showed a progressive effect with a longer duration of heat leading to increasingly more mealiness. 'Elegant Lady', however, was more susceptible to the disorder and most of the fruit were mealy even in the control treatment following 2 weeks storage. Analysis of the overall data indicated that development of mealiness was significantly affected by heat in a continuous, exponential manner ($P \leq 0.01$).

Exopolygalacturonase activity decreased due to HTFA treatment in all three cultivars (Fig. 3). The overall decrease, however,

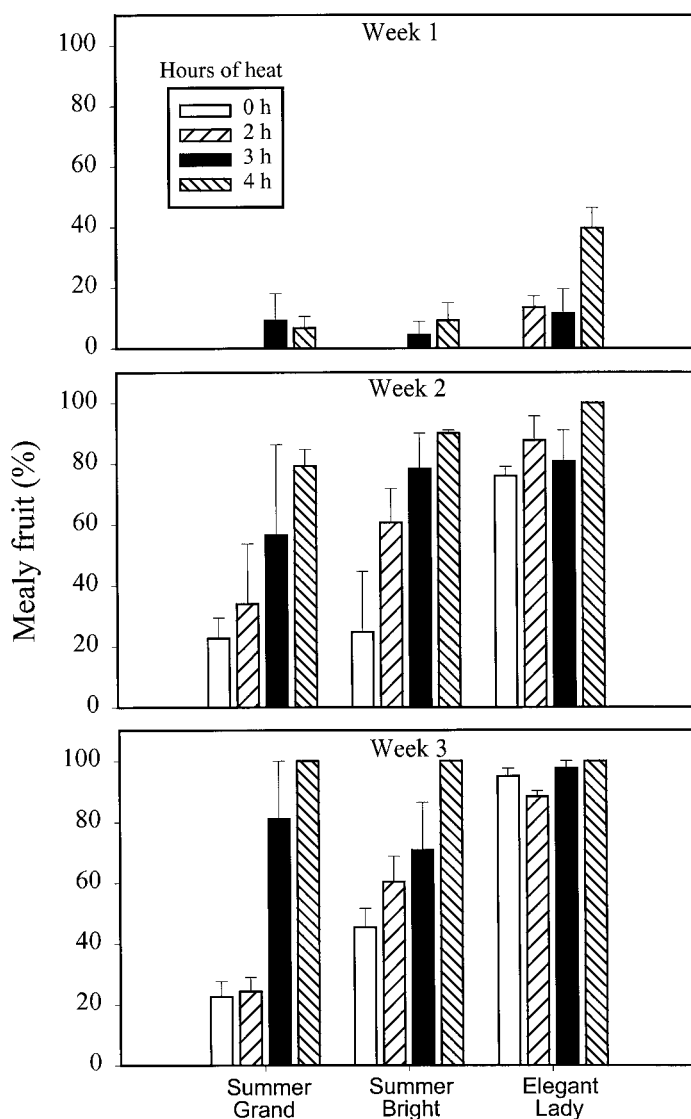


Fig. 2. Percentage of mealy fruit of three peach or nectarine cultivars following HTFA treatment and subsequent cold storage for 1, 2, or 3 weeks at 5 °C followed by 3 d at 22 °C. Each value represents a mean \pm SE of three replications with 15 to 20 fruit per replication.

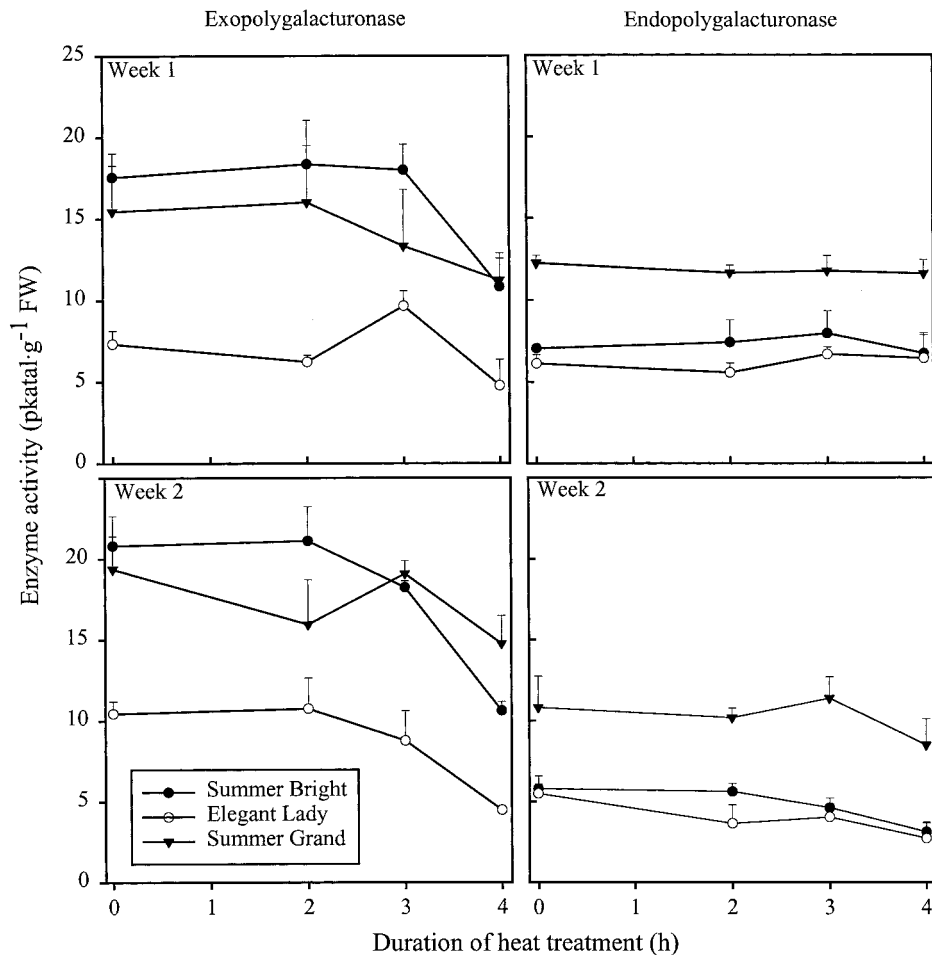


Fig. 3. Activities of exo- and endopolygalacturonase from three peach or nectarine cultivars measured after various durations of HTFA-treatment and 1 or 2 weeks of cold storage at 5 °C followed by 3 d at 22 °C. Three individual fruit were assayed for each of the three replications. Each symbol represents the mean \pm SE of nine fruit.

was only significant ($P \leq 0.001$) after 4 h of treatment following either 1 or 2 weeks storage. Exopolygalacturonase activity was similar in 'Summer Bright' and 'Summer Grand' nectarines and greater ($P \leq 0.001$) than that of 'Elegant Lady' peaches.

The effect of treatment on endo-PG activity (Fig. 3) was less pronounced than that observed for exo-PG and was not statistically significant ($P \leq 0.09$). 'Summer Bright' and 'Elegant Lady' had nearly identical activities, while that of 'Summer Grand' was greater ($P \leq 0.001$) after both 1 and 2 weeks storage. Overall,

activity following 1 week of storage was greater than after 2 weeks ($P \leq 0.001$).

Activities of exo- and endo-PG in individual heat-treated fruit were lower in the mealy regions than in the juicy regions on a fresh weight (FW) basis (Table 1). Part of the large decline in PG activity was likely related to the >50% loss in protein in the mealy region as compared to the juicy (Table 1). In fact, when calculated on a protein basis, exo-PG activity was not statistically different in juicy as compared to mealy tissue. Pectinmethylesterase activity, however, was very similar in mealy and juicy regions on a FW basis and had greater activity in the mealy regions on a protein basis (Table 1).

Intermittent warming effectively reduced development of mealiness in both control and HTFA-treated fruit following 2 weeks storage (Fig. 4). The amount of reduction in the control was nearly 60% but in the HTFA-treated fruit was 32%. Even though the percentage of mealy fruit increased with longer storage (3 weeks), the effect of intermittent warming was still markedly greater for the control fruit following this amount of storage.

Discussion

Peaches and nectarines treated with HTFA developed mealiness sooner and to a greater degree than nonheated fruit. The effect was obvious even in 'Summer Grand' nectarines, a cultivar characterized previously as being relatively resistant to the development of mealiness (Crisosto et al., 1999). In the case of 'Summer Grand' and 'Summer Bright' nectarines the effect was additive in that an increase in the duration of heating led to an additional increase in the percentage of mealy fruit, while the majority of 'Elegant Lady' peaches, due to a greater susceptibility to the disorder, were mealy after 2 weeks of storage in all of the treatments and thus did not show a definitive effect of heat after that point.

Promotion of chilling-injury symptoms by heat is in contrast to many reports of heat-induced resistance to chilling injury (Florissen et al., 1996; Lurie et al., 1997; Woolf, 1997). In fact, it

Table 1. Pectolytic activity and protein concentration from juicy or mealy sectors of 'O'Henry' peach tissue.

Variable		Juicy ^z	Mealy	$P > F^y$
Exopolygalacturonase	(pkatal·g ⁻¹ fresh weight)	18.89 (± 3.12)	6.69 (± 2.42)	0.0019
	(pkatal·mg ⁻¹ protein)	236.81 (± 54.66)	150.04 (± 47.23)	0.2084
Endopolygalacturonase	(pkatal·g ⁻¹ fresh weight)	17.82 (± 2.28)	2.55 (± 0.77)	0.0003
	(pkatal·mg ⁻¹ protein)	233.70 (± 54.91)	60.29 (± 14.51)	0.0265
Pectinmethylesterase	(nkatal·g ⁻¹ fresh weight)	0.048 (± 0.01)	0.046 (± 0.01)	0.8500
	(nkatal·mg ⁻¹ protein) ^x	888.87 (± 146.50)	1650.38 (± 212.93)	0.0166
Protein	(mg·mL ⁻¹)	0.190 (± 0.04)	0.079 (± 0.01)	0.0306

^zValues are based on the mean of individual assays from seven separate fruit. Numbers in parenthesis are SEs.

^yAnalysis was performed using paired *t* test to determine the level of significance.

^xCalculated on the basis of protein concentration in the assay extract.

has been frequently demonstrated that one stress can often confer a degree of resistance against another. Generally, however, such treatments involving heat are not of the same severity as was used in this work in terms of temperature and duration of treatment. Too severe of a prestress may act as a damaging factor and overwhelm any positive benefit that is conferred by the heat treatment

A characteristic effect of heat upon fruit is an impairment or alteration of the ripening process. The outward effects include inhibition of fruit softening (Eaks, 1978; Porritt and Lidster, 1978), alteration of pigmentation change (Seymour et al., 1987), reduced ethylene production (Chan, 1986; Ketsa et al., 1999) and, in some cases, changes in titratable acidity. The effect on fruit softening is especially pertinent to this study as it is believed that one possible cause of mealiness is a chilling-induced alteration in the activity of PG (Buescher and Furmanski, 1978), an enzyme that is generally thought to be involved in the softening process. Polygalacturonase activity increases greatly during the ripening of many fruit, and is associated with changes in the structure of pectin and fruit softening (Fischer and Bennett, 1991). Storage of tomatoes (*Lycopersicon esculentum* Mill.) at 33 °C has been reported to prevent fruit softening, presumably due to the observed lack of PG activity (Ogura et al., 1975b; Yoshida et al., 1984). During ripening of heat-treated tomatoes at room temperature, however, the same authors found that the PG activity would recover, although to only one-third of the amount found in normally ripened fruit. In agreement with these previous studies, our research also found heat treatment to be inhibitory to PG activity. It was apparent that a decline in exo-PG occurred when heat was applied for 4 h and, in some cases, as brief as 3 h (Fig. 3). A reduction in endo-PG activity was also observed, although it was not as pronounced as that for exo-PG. Endo-PG has been reported previously to be the PG enzyme most closely associated with chilling injury and the development of mealiness (Artés et al., 1996).

Comparison of mealiness and PG activity data indicates that the fruit often began to show enhanced mealiness at a duration of heat that did not necessarily alter PG activity. This could be an indication that heat may be acting to enhance mealiness in a manner more complex than the inhibition of a single enzyme. Another possibility is associated with the way in which the disorder expresses itself. Typically, fruit that became mealy were found not to be totally

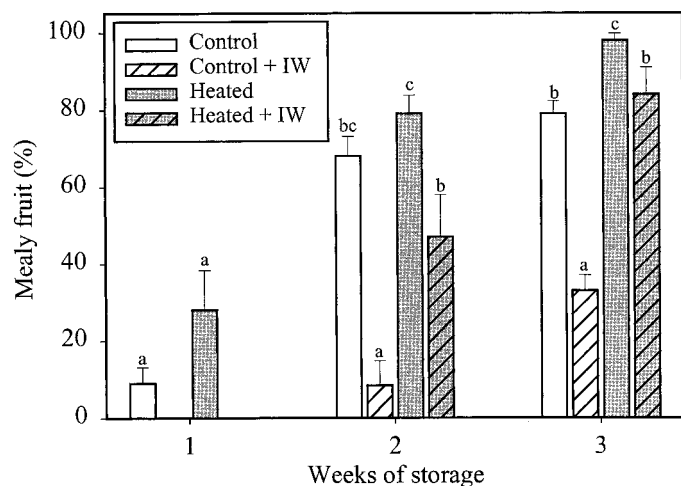


Fig. 4. Influence of intermittent warming (IW) on the mealiness of heat-treated or control 'September Sun' peaches following 1, 2, or 3 weeks storage at 5 °C. Each value represents the mean \pm SE of four replications with 15 to 20 fruit per replication. Mean separation by Duncan's multiple range test, $P \leq 0.05$.

mealy, but very often had regions, such as the shoulders, that were mealier than others. Orientation of the fruit in relation to the heat source and uneven heating of the fruit were determined not to be a factor in determining where mealy regions would develop (data not presented). In a random sampling from the equatorial regions of the fruit, such as was done for this experiment to determine PG activity, it is possible that mealy regions may have been missed and the measured activity not totally reflect the fruit as a whole. In fact, in a later experiment, development of the mesocarp tissue into easily observed regions that were juicy or mealy was exploited to enable a clearer comparison of the two tissue types (Table 1). Differences in both exo- and endo-PG activity between the regions were much more pronounced than that observed in our previous experiment in which we took samples at random. Endo and exo-PG were reduced in activity by 86% and 65%, respectively, in mealy tissue.

Although reports have differed on the effect of cold storage on PME (Artés et al., 1996; Ben-Arie and Sonogo, 1980; Von Mollendorf and De Villiers, 1988), this enzyme is thought to play a role in the development of mealiness by demethylating pectin which, in conjunction with low PG activity, leads to formation of a high molecular weight, low methoxy pectin that binds water and forms an insoluble gel (Ben-Arie and Sonogo, 1980). Our results indicated that on a FW basis there was no significant difference in PME activity between mealy and juicy regions (Table 1) and, together with our PG data, support the idea that mealiness is caused by an imbalance in the activities of these two enzymes, being due primarily to a loss of PG activity (Artés et al., 1996). The higher PME activity of mealy fruit on a protein basis was likely due to the reduction in extractable soluble protein in the mealy tissue. Since all samples were from peaches that received heat, the direct effect of heat on PME activity is unknown.

Intermittent warming is effective in preventing or slowing development of mealiness (Anderson, 1982; Dawson et al., 1995). This technique was used in the present study as a means of examining if mealiness due to heat treatment and subsequent cold storage could be successfully minimized by intermittent warming during storage. Our results found that intermittent warming was far less effective in preventing mealiness when a heat treatment was superimposed upon cold storage (Fig. 4) and, at least with the particular HTFA-treatment and cultivars we were using, would not be useful in preventing the disorder.

In the present study it was found that heat treatment, such as that which would be performed in a quarantine insect disinfestation treatment, can be detrimental to the market quality of peaches and nectarines by accelerating and enhancing development of mealiness. This is a very serious side effect of heat treatment that must be considered carefully if quarantine treatments involving heat are to be successfully developed for peaches and nectarines. Our results further indicate that a possible mode of action of heat is through an inhibition of PG, leading to formation of an insoluble gel and a mealy texture in the fruit. The imperfect correlation between PG activity and the development of mealiness that we observed, however, must also invite consideration that other factors may be involved in the enhancement by heat of this disorder.

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