Heat Treatments Delay Ripening and Postharvest Decay of Strawberry Fruit

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Fully red strawberries (*Fragaria* × *ananassa* Duch. cv. Selva) were treated for 1−5 h at temperatures ranging from 39 to 50 °C. After treatments, fruits were placed at 0 °C overnight and then held at 20 °C for 3 days. Most of the heat treatments improved strawberry shelf life, with best results obtained for fruit heated at 42 °C and 48 °C for 3 h. Heat treatments prevented fungal development and decreased the number of damaged fruits. These treatments were then used to treat strawberries at 50−75% red to analyze effects on firmness, surface color, anthocyanin content, PAL activity, polypeptide composition, and protein synthesis. Softening rate and external color development was less in fruit heated at 48 °C compared to the control, while treatment at 42 °C did not significantly affect these parameters. However, both treatments reduced anthocyanin accumulation and PAL activity relative to the controls. In addition, treatment at 48 °C for 3 h reduced protein synthesis, but this effect was partially reversed after holding fruits at 20 °C for 48 h. Heat treatments (42 and 48 °C) led to the accumulation of five heat shock proteins of 88, 76.5, 69, 19, and 17 kDa. In addition, treatment at 42 °C induced the synthesis of a 22 kDa polypeptide that was not observed in the 48 °C treatment. Fruits treated at 48 °C continued the synthesis of 69, 19, and 17 kDa polypeptides even after 48 h at 20 °C. According to these results, the lower fungal development and the slower ripening rate shown by heat-treated fruits suggest that this physical method could be useful to extend the postharvest shelf life of strawberry.

Keywords: *Strawberry; heat treatment; ripening; postharvest decay; heat shock protein; protein synthesis*

INTRODUCTION

Some chemical treatments applied to fruits to prevent insect attack or prolong their postharvest shelf life are potentially dangerous to human beings. For this reason, some physical methods are being extensively studied as substitutes for current chemical methods in commercial use (Paull, 1990; Klein and Lurie, 1991). Examples of physical methods are high- or low-temperature treatments and irradiation and use of modified or controlled atmospheres. High-temperature treatments can control insect pests, prevent pathogen infection, induce resistance to chilling injury, slow fruit ripening, and extend postharvest shelf life (Paull, 1990; Klein and Lurie, 1991). Application of thermal treatments reduced the softening rate of apples (Lurie and Klein, 1990), tomatoes (Yoshida et al., 1984), and pears, plums, and avocados (Klein and Lurie, 1991). Besides, thermal stress affects the capacity of biological systems to synthesize proteins, resulting in more or less synthesis of present proteins and the synthesis of a new set of specific proteins termed "heat shock proteins" (HSP) (Brodl, 1989).

Most previous works have studied heat treatments on climacteric fruits, including tomato, apple, avocado, peach, papaya, pear, and banana (Biggs et al., 1988; Paull, 1990; Paull and Chen, 1990; Lurie and Klein, 1990), with little work on the application of heat treatments to nonclimacteric fruits, such as strawberry. Regarding this, Couey and Follstad (1966) successfully controlled postharvest decay by applying humid air at 44 °C to five strawberry cultivars (Fresno, Solana, Lassen, Shasta, and Z5A). García et al. (1995) reduced postharvest losses by immersing strawberries (cv. Tudla) in water at 45 °C for 15 min. In contrast, Yoshikawa et al. (1992) treated Chandler strawberries with humid air at 43 and 46 °C for 80 min and found severe damage in the fruits. These contradictory results could be due to cultivar-dependent responses of strawberry to heat treatments, as has been described for other fruits (Paull, 1990; Kim et al., 1993).

Strawberry fruit shows an important decrease of firmness and color change during ripening. A great loss of chlorophylls and synthesis and accumulation of anthocyanin occurs in this period (Woodward, 1972; Given et al., 1988a,b; Cheng and Breen, 1991). Anthocyanins are flavonoids synthesized from the aromatic amino acid phenylalanine, and one of the key enzymes for their synthesis is phenylalanine ammonia−lyase (PAL) (Hahlbrock, 1981). Moreover, other authors have revealed a close relationship between the enzymatic activity of PAL and anthocyanin content during strawberry fruit ripening (Given et al., 1988b; Cheng and Breen, 1991). Therefore, parameters such as fruit firmness, surface color, anthocyanin content, and PAL activity could be used to evaluate the effect of heat treatments on strawberry fruit ripening during postharvest storage.

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S0021-8561(97)00033-2 CCC: $14.00

Strawberry shelf life may be improved by an appropiate thermal treatment that could be used instead of fumigation to allow a more advantageous usage of this fruit in the commercial chain. A necessary step to achieve such a goal is to improve knowledge on the biochemical and physiological alterations caused by heat treatments.

The aim of the present work was to analyze the effect of high-temperature treatments on quality and ripening parameters of strawberry cv. Selva. To this end, fruits were treated under different time−temperature combinations to evaluate the effectiveness of the treatments to reduce fruit decay at 20 °C. Once the more effective treatments were chosen, we analyzed the effect on fruit firmness, development of surface color, anthocyanin content, PAL activity, polypeptide composition, and protein synthesis.

MATERIALS AND METHODS

Plant Material. Strawberries (*Fragaria* x *ananassa* Duch. cv. Selva) grown in greenhouses were harvested at different ripening stages and classified by their surface color as 50−75% red or 100% red. The fruits at 50−75% red were treated for 10 min with a solution of NaClO (1.1 g of Cl/L), thoroughly rinsed with tap water for 15 min, and then drained on filter paper. Fruits at 100% red were only rinsed with tap water during 15 min and then drained on filter paper. Fruits harvested at 100% red were used to evaluate the effect of heat treatments on external appearance. Fruits harvested at 50−75% red were used to measure the influence of selected treatments on firmness, surface color, anthocyanin content, polypeptide composition, and protein synthesis.

Heat Treatments. Thermal treatments were applied to fruits contained in plastic trays covered, but not sealed, with a PVC film (15 μm thickness) to retard water loss. The fruit weight loss was monitored, and it did not exceed 2% after any treatment. Each tray had 10−12 fruits of the same ripening stage, and two or three trays, selected at random, were used for each heat treatment. Heat was applied by leaving the trays in an air oven set at the following temperature−time conditions: 39 °C, 5 h; 42 °C, (1, 3, and 5 h); 48 °C, (1, 2, 3, and 4 h); 50 °C, (2, 4, and 5 h). Once treatments were over, trays were placed at 0 °C overnight and then held at 20 °C for 3 days. Corresponding controls were not thermally treated but directly brought to 0 °C and then left at 20 °C for 3 days. Samples were taken at the beginning of the experiment, after the treatments, and after 24, 48, and 72 h of being exposed to 20 °C. When it was necessary, samples were taken and stored at −60 °C until use.

Evaluation of the External Appearance. The external appearance of fully red strawberries (heat treated and controls) was followed during the incubation at 20 °C for 3 days. The presence of exudate (juice leak), macroscopic fungal growth, and injuries on the fruit surface were visually evaluated. Fruits presenting any of those characteristics were considered as damaged fruits.

Determination of Fruit Firmness. Strawberries at 50−75% red ripening stage were treated at 42 and 48 °C for 3 h, and their firmness was measured during incubation at 20 °C for 3 days. Firmness values were determined in an Instron Model 1011 fitted with an 8 mm diameter flat probe. The probe descended toward the sample at 20 mm/min during the tests. The value recorded was the maximum force (*N*) reached during tissue breakage. The firmness of each fruit was measured twice on opposite sides of the central zone of the 25−30 berries used per treatment.

Determination of Surface Color. Surface fruit color is a commercial indicator used to determine strawberry ripeness. Strawberries at 50−75% red ripening stage were treated at 42 and 48 °C for 1 and 3 h, and their surface color was followed during incubation at 20 °C for 3 days. The objective parameters of surface color (L, a and b) were measured with a HunterLab colorimeter in five zones of each fruit. A total of 30 berries were used in each treatment.

Extraction and Determination of Anthocyanins. Strawberries at 50−75% red ripening stage were treated at 42 and 48 °C for 3 h. Samples (10 fruits) were taken and stored at −60 °C before, immediately after the thermal treatments, and during the 3 days of incubation at 20 °C. Determination of anthocyanins was done using fruits frozen at −60 °C by crushing them in a refrigerated mill (Tekmar, Model A-10). About 400 mg of the resultant powder was poured in 10 mL of a HCl−methanol solution (1% v/v) and held at 0 °C for 10 min. The resultant slurry was centrifuged at 1500*g* for 5 min at 4 °C. Absorbance at 515 nm was measured on the supernatant, and the anthocyanin content, expressed as pelargonidin 3-glucoside, was calculated by using $E_{\text{molar abs}} = 36\,000\ M^{-1}\cdot cm^{-1}$ (Woodward, 1972).

Extraction and Activity of Phenylalanine Ammonia-Lyase (PAL). Strawberries at 50−75% red ripening stage were treated at 42 and 48 °C for 3 h. Samples (10 fruits) were taken, and PAL activity was measured before, immediately after the thermal treatments, and during the 3 days of incubation at 20 °C. Determination of PAL activity was done as follows: the fruits were cut in small pieces, and about 15 g of fruits were homogenized in an Omnimixer at 4 °C with four volumes of buffer of the following composition: 0.1 M $Na_2B_4O_7\cdot 10H_2O$, 5 mM 2-mercaptoethanol, 2 mM EDTA, 30 g/L of polyvinylpolypyrrolidone (PVPP), pH 8.8. The mixture was left for 1 h at 4 °C under magnetic stirring and then centrifuged at 10000*g* for 20 min at 4 °C. Enzymatic activity was measured in the supernatant by the method reported by Zucker (1965), using the following reaction mixture: 0.03 M $Na_2B_4O_7\cdot 10H_2O$ (pH 8.8), 0.01 M L-phenylalanine, 1 mL of enzymatic extract, in a total volume of 3 mL. This mixture was incubated at 30 °C, and the reaction evaluated through the increase in optical density at 290 nm, caused by production of *trans*-cinnamic acid. The enzymatic activity unit (kat) was defined as the amount of enzyme required to produce 1 mol of *trans*-cinnamic acid per second under the above-mentioned conditions. The specific enzymatic activity was calculated as enzymatic activity per mg of protein.

Determination of Proteins. The protein concentration of PAL enzymatic extracts were determined by the modified Lowry method (Potty, 1969) using a wavelength of 750 nm.

Extraction of Polypeptides. Strawberries at 50−75% red ripening stage were treated at 42 and 48 °C for 3 h. Samples (10 fruits) were taken and stored at −60 °C before, just after the treatments, and during the incubation at 20 °C for 3 days. Polypeptides were extracted as follows: strawberries frozen at −60 °C were crushed in a refrigerated mill, and about 5 g of the resultant powder was stirred in 20 mL of extraction buffer for 90 min at room temperature. The composition of the buffer used was as follows: 50 mM tris-base; 2% (w/v) sodium dodecyl sulfate (SDS); 2% (v/v) mercaptoethanol; 1 mM EDTA; 5% (w/v) sucrose; 6 g/L PVPP; pH 7.0. The suspension so obtained was centrifuged at 9200*g* for 20 min at 20 °C, the supernatant was retained, and the proteins were precipitated by adding solid trichloroacetic acid (TCA) to obtain a 10% (w/v) concentration. The mixture was stirred for 2 min, held overnight at 0 °C, and then centrifuged at 9200*g* for 30 min at 4 °C and the supernatant discarded. The precipitate was washed twice with 80% (v/v) acetone to remove the remaining acid. In turn, any acetone remaining was eliminated by vacuum evaporation, and the resultant pellet was dissolved in 500 μL of 8 M urea, followed by 500 μL of sample buffer (62.5 mM tris-base; 2% (w/v) SDS; 5% (v/v) mercaptoethanol; 25 mM EDTA; 15% (w/v) sucrose; pH 6.8). The suspension was then centrifuged and the supernatant so obtained stored at −60 °C until use.

Electrophoresis under Denaturing Conditions (SDS−PAGE). Polyacrylamide gels with SDS were prepared according to the system of discontinuous buffers of Laemmli (1970). The gels were prepared in a linear gradient of acrylamide concentration (10−18% w/v) and the electrophoresis carried out at constant intensity (15 mAmp/plate in the stacking gel and 25 mAmp/plate in the separating gel). After the electrophoresis, gels were stained with 0.1% (w/v) Coo-

massie-Blue R-250. The presence of high concentrations of urea, SDS, and mercaptoethanol prevented the protein amount determination (Lowry et al., 1951; Bradford, 1976). Thus, instead we used the criterion of Veluthambi and Poovaiah (1984), and trial runs were conducted to determine the sample volumes leading to comparable intensities for common polypeptide bands.

[^{35}S]Methionine Labeling. Strawberries at 50−75% red ripening stage were used in two kinds of labeling experiments.

(a) Heat Treatments on Fruit Disks. Fruits were cut transversely at midlength into 1−2 mm thick tissue slabs or disks and 12 of them were used in each treatment. Pieces were weighed and placed in Petri dishes containing 15 mL of the following buffer: 60 mM citric acid; 74 mM Na_2HPO_4; 2% (v/v) dimethyl sulfoxide (DMSO); pH 4.0. Then, 20 μL of an aqueous solution of [^{35}S]methionine (specific activity > 600 Ci/mmol) was added. The concentration of the solution was selected to deposit 10−15 μCi of [^{35}S]methionine in each disk. The incubation with the radioactive label was maintained at 42 or 48 °C for 3 h, after which time the disks were frozen at −60 °C until use. Control fruit disks were labeled at 20 °C for 3 h.

(b) Heat Treatments on Whole Fruits. Fruits were treated at 42 or 48 °C for 3 h, while control fruits were held at 20 °C for 3 h. Some control and treated fruits were used for labeling immediately after incubation. The remaining fruits were held at 20 °C for 48 h and then used for labeling. In all cases, fruit disks were obtained and labeled as described in (a), except that the incubation with [^{35}S]methionine was done at 20 °C for 3 h, after which time the disks were frozen at −60 °C until use.

Frozen disks were crushed with a mortar, and polypeptides were extracted by the above-mentioned method. Two aliquots of 1 mL were taken from the extract and poured on 10 mL of TCA 10% (w/v) to precipitate the proteins, held overnight at 0 °C, and then filtered through 0.45 μm filters, and the quantity of radioactivity incorporated to the proteins was determined by a scintillation counter (Rackbetta 1214, Pharmacia). The remaining part of the extract was precipitated as usual with solid TCA and then analyzed by denaturing electrophoresis and autoradiography.

Electrophoresis and Autoradiography. The extracts were analyzed by denaturing electrophoresis (SDS−PAGE) as described previously. Gels were stained with Coomassie-Blue R-250 as usual and then wrapped with a water-permeable plastic film, oven-dried at 37 °C, and put in contact with a Kodak film X-OMAT-XK-1 for 2−3 weeks at −60 °C.

Experimental Design. All experiments were done at least in triplicate. The results of fruit firmness, surface color, anthocyanin content, PAL activity, and [^{35}S]methionine incorporation were analyzed by ANOVA and the means compared by the LSD test at a significance level of 0.05.

RESULTS AND DISCUSSION

External Appearance. Fruits in commercial ripening stage (100% red) were thermally treated and then held at 20 °C, and their external appearance was evaluated after 24, 48, and 72 h (Table 1). Observations made after 24 h at 20 °C are not shown since we did not detect any difference in general appearance between untreated (control) and treated fruits.

The percentage of damaged control fruits was 50% after 48 h and increased to 90−100% after 72 h. Up to 25% of these fruits had fungal growth after 48 h at 20 °C, which increased to 50% of the berries after 72 h. In addition, abundant exudate (juice leak) and surface injuries were observed after 72 h.

The damage exhibited by fruits treated at 39 °C for 5 h was similar to that of the controls, but without any fungal growth even after 72 h. Fruits treated at 42 °C for 1 h had similar damage compared to corresponding controls. Moreover, heat-treated fruits had more fungal growth after 72 h. In contrast, berries given the longer heat treatments (3 or 5 h at 42 °C) had less damage and no fungal growth relative to controls. Fruits treated at 48 °C for 1 or 4 h had damage similar to that of the controls. In turn, treatments for 2 and 3 h at 48 °C led to fruits with no fungal growth and less number of damaged fruits. Finally, treatments performed at 50 °C (2, 4, and 5 h) induced considerable fruit damage, with greater softening, stronger fungal growth, and more surface injuries than the corresponding controls. Our results are similar to those reported by García et al. (1995), who used a different heating method: they treated strawberries in water at different temperatures for 15 min and observed severe fruit damage at 55 °C and best results at 45 °C.

Table 1. Evaluation of External Appearance of Strawberry Fruits Treated at Different Temperatures and Then Incubated at 20 °C

treatment (temp (°C), time (h))	48 h at 20 °C			72 h at 20 °C		
	fungal			fungal		
	exudate	growth[a]	damage[a]	exudate	growth[a]	damage[a]
control	regular	+	++	abundant	++	++++
39, 5	low	−	++	regular	−	+++
42, 1	regular	+	++	abundant	++++	++++
42, 3	very low	−	+	low	−	++
42, 5	low	−	+	low	−	++
48, 1	regular	+	++	abundant	+++	+++
48, 2	none	−	+	regular	−	++
48, 3	low	−	+	regular	−	+
48, 4	very low	−	+++	regular	+	+++
50, 2	none	++	+++	abundant	+++	++++
50, 4	none	++	+++	abundant	+++	++++
50, 5	ND	++	ND	abundant	+++	ND

[a] Key: −, absence; +, presence in 0−25% of fruits; ++, presence in 25−50% of fruits; +++, presence in 50−75% of fruits; ++++, presence in 75−100% of fruits; ND, not determined.

Results showed in the present work indicate that heat treatments at 42 and 48 °C for 3 h reduced postharvest decay and fungal growth in ripe strawberry fruits. Treatments done at temperatures above 48 °C were no good, while those at 48 °C or less showed a time-dependent response. As best results were found by applying heat treatments at 42 and 48 °C for 3 h, both conditions were chosen to analyze the effect of heat treatments on parameters closely associated to fruit ripening (firmness, surface color, anthocyanin content, polypeptide composition, and protein synthesis).

Fruit Firmness. Control and treated fruits used to evaluate firmness (50−75% red) did not show exudate, injuries, or fungal growth during the incubation at 20 °C. In control fruits and in those treated at 42 °C for 3 h, firmness decreased significantly over the first day of incubation at 20 °C, and the rate of softening slowed down from then on (Figure 1). Thus, treatment at 42 °C for 3 h did not modify softening rate in strawberry fruits. In contrast, fruits treated at 48 °C for 3 h were more resistant to penetration than the corresponding controls, indicating that this treatment retards fruit softening.

It was reported that heat treatments can reduce the softening rate of apples (Lurie and Klein, 1990), tomatoes (Yoshida et al., 1984), and pears, plums, and avocados (Klein and Lurie, 1991). Until now, however, contradictory results have been reported on the effect of heat treatments on strawberry firmness. Yoshikawa et al. (1992) reported that fruits treated with moist air at 37−46 °C were slightly softer than controls. However, García et al. (1995) submerged fruits in water at 45 °C for 15 min and concluded that firmness was enhanced. The diverse responses of fruits to the above-mentioned thermal treatments might be caused by

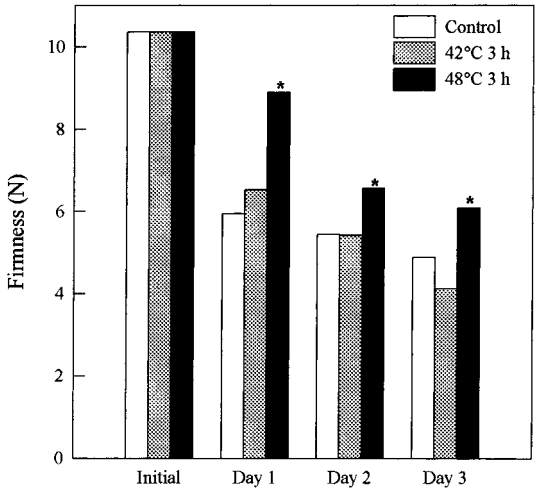

Figure 1. Changes in fruit firmness of strawberries (50−75% red) nontreated and heat treated during 3 days of storage at 20 °C. Key: white, control; shaded, 42 °C, 3 h; black, 48 °C, 3 h. The symbol * indicates significant differences ($\alpha = 0.05$) in relation to control.

differences among heating procedures, cultivars, and ripening stages.

External Color Development. In experiments carried out with fruits of commercial ripening stage, we observed less surface color development in fruits treated at 39, 42, or 48 °C (data not shown). Thus, for better color evaluation, it was decided to test strawberries in an earlier ripening stage (50−75% red).

The parameter *L* is a measure of fruit brightness, whereas the *a*/*b* ratio is usually used to follow the color evolution in fruits changing from green to red over their ripening process (Babbit et al., 1973; Purvis and Barmore, 1981).

The average value of the *a*/*b* ratio increased during the incubation period at 20 °C, though this increase was lower in treated than in control fruits (Figure 2). Fruits treated at 48 °C for 3 h had *a*/*b* values significantly lower than those of corresponding control fruits during the incubation. Treatment at 48 °C for 1 h was effective in preventing red color development only during the first 2 days, while that at 42 °C for 3 h was so only for the first day. Finally, the differences observed between fruits treated at 42 °C for 1 h and the controls were not significant over the entire incubation period. Brightness of strawberry fruit decreases during the postharvest period, and this change can be followed by measuring the parameter *L* (Collins and Perkins-Veazie, 1993). Fruits treated at 48 °C for 3 h were brighter (higher *L* value) than controls during incubation at 20 °C. Fruits treated at 48 °C for 1 h and 42 °C for 3 h showed higher *L* values only during the first day, while that at 42 °C for 1 h did not show significant differences in any of the three incubation days.

The results previously discussed do not agree with the conclusions of García et al (1995), who did not detect any color differences between heat-treated and control fruits when using strawberries cv. Tudla.

Anthocyanin Content. Anthocyanin content was measured as indicated in the Materials and Methods. Immediately after heat treatments, the average anthocyanin levels of treated fruits were higher than the initial values, though not enough to be statistically significant (Figure 3a). Although anthocyanin content did increase in all fruits during incubation at 20 °C, values for thermally treated fruits were significantly

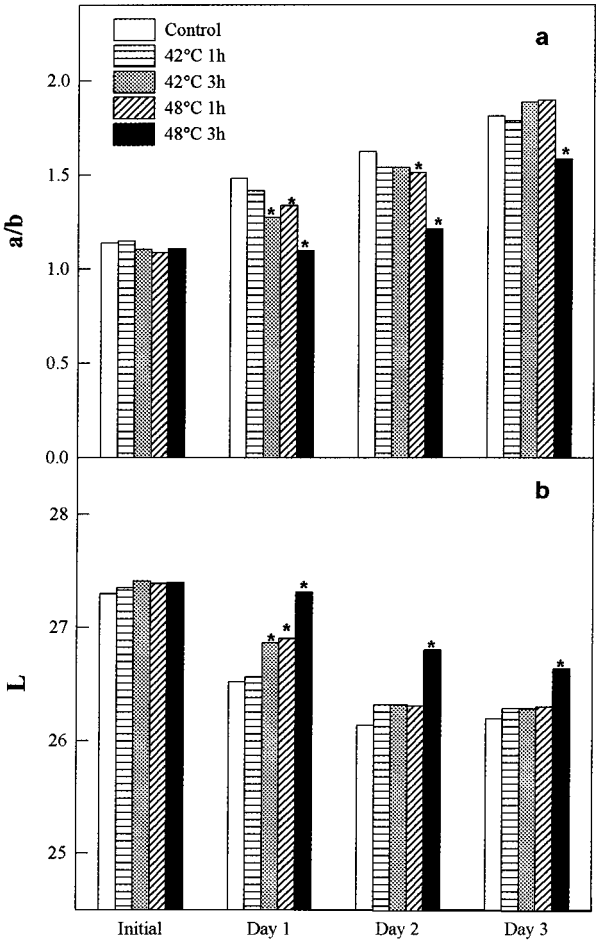

Figure 2. Changes in external color parameters of strawberries (50−75% red) nontreated and heat treated during 3 days of storage at 20 °C. (a) Evolution of *a*/*b* ratio. (b) Evolution of *L* parameter. Key: white, control; horizontal hatching, 42 °C, 1 h; shaded, 42 °C, 3 h; diagonal hatching, 48 °C, 1 h; black, 48 °C, 3 h. The symbol * indicates significant differences ($\alpha = 0.05$) in relation to control.

lower than those of corresponding controls over the entire incubation period. Moreover, anthocyanin content of fruits treated at 48 °C was significantly lower than that of fruits treated at 42 °C.

The lower accumulation of anthocyanins induced by the 48 °C, 3 h treatment agrees with the abovementioned delay of surface color development. On the other hand, the treatment at 42 °C for 3 h reduced anthocyanin accumulation, though this effect could not be detected by the corresponding surface color measurements. In this sense, as the determination of anthocyanin content uses a representative sample of the whole fruit mass, it results in a more sensitive measure than surface color evaluation to detect effects of thermal treatments.

PAL Activity. PAL activity was measured in control and treated fruits (42 °C for 3 h and 48 °C for 3 h), in parallel with the determination of anthocyanin content. It was observed that, immediately after treatment at 42 °C for 3 h, the specific PAL activity was significantly higher than that of initial fruits (Figure 3b). In contrast, treatment at 48 °C for 3 h induced a significant decrease of such activity. During incubation at 20 °C, PAL activity increased continuously in both control and treated fruits. However, treated fruits presented a lower activity compared to controls, this difference being more pronounced in the 48 °C treatment. Thus, the

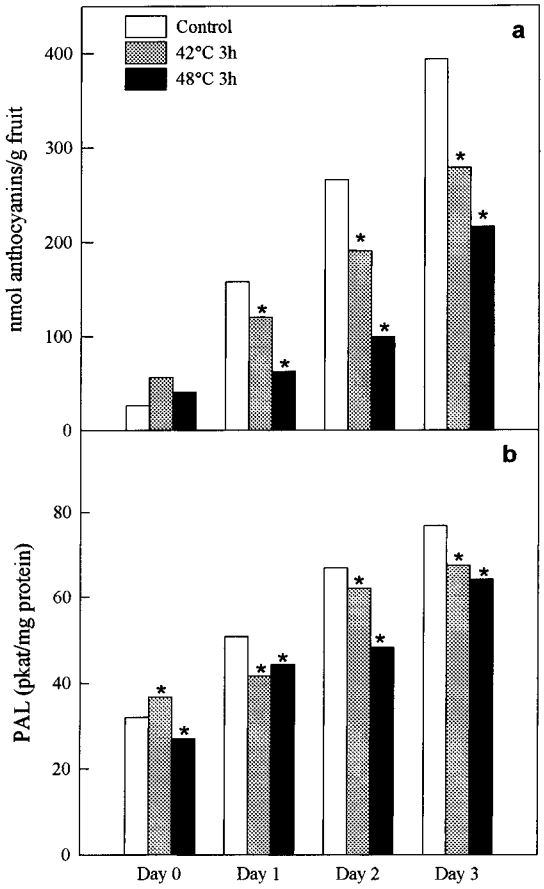

Figure 3. Changes in anthocyanin content and PAL activity of strawberries (50-75% red) nontreated and heat treated during 3 days of storage at 20 °C. Columns of day 0 indicate the anthocyanin content and PAL activity of fruits in initial conditions (control) and immediately after treatments (42 °C, 3 h and 48 °C, 3 h). (a) Anthocyanin content. (b) PAL activity. Key: white, control; shaded, 42 °C, 3 h; black, 48 °C, 3 h. The symbol * indicates significant differences ($\alpha = 0.05$) relative to control.

delayed surface color development and anthocyanin accumulation observed in thermally treated fruits can be attributed to a delay in PAL activity increase. In turn, provided that PAL is synthesized "de novo" during strawberry fruit ripening (Given et al., 1988c) and that heat treatments reduce protein synthesis (Brodl, 1989; Lurie and Klein, 1990), it is possible that these treatments affect PAL activity by slowing down PAL synthesis "de novo".

SDS−PAGE of Polypeptides. The possible effect of heat treatments on polypeptide composition was analyzed. Polypeptide profiles of control and treated fruits showed numerous species with molecular mass values ranging from 10 to 95 kDa, being similar to those described previously for this strawberry variety at ripe stage (Civello et al., 1996). Profiles obtained immediately after the treatments were similar to the initial ones (data not shown). However, variations in at least three species were detected during the incubation period at 20 °C. Treated fruits at 42 and 48 °C showed an increase in polypeptides of 19 and 69 kDa, especially in the first 2 days. The accumulation of a 40.3 kDa polypeptide was delayed in fruits heated at 48 °C but not in those treated at 42 °C.

Modifications in polypeptide profile of thermally treated fruits have been previously reported for papaya by Paull and Chen (1990). In such work, they have observed accumulation of four species (70, 30, 20, and

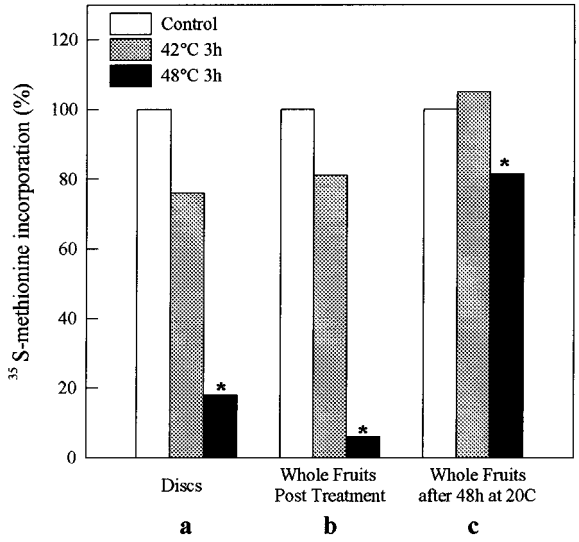

Figure 4. Incorporation of [^{35}S]methionine into proteins. Values plotted on the graph represent the percent of incorporation in relation to control (100%). (a) Fruit disks incubated and labeled at different temperatures. (b) Whole fruits treated at different temperatures. Disks were obtained immediately after treatments and labeled at 20 °C during 3 h. (c) Whole fruits treated at different temperatures and stored at 20 °C during 48 h. Then, disks were obtained and labeled at 20 °C during 3 h. Key: white, 20 °C, 3 h (control); shaded, 42 °C, 3 h; black, 48 °C, 3 h. The symbol * indicates significant differences ($\alpha = 0.05$) in relation to control.

19 kDa) and disappearance of seven polypeptides after the heat treatment. In this regard, two of the species we found in strawberry (69 and 19 kDa) have molecular masses similar to those reported in papaya (70, 20, and 19 kDa).

Protein Synthesis. *(a) Heat Treatments on Fruit Disks.* We measured the amount of [^{35}S]methionine incorporated into proteins by incubating fruit disks directly with the radioactive label at the temperature of the treatment (Figure 4a). It can be observed that protein synthesis at 48 °C decreased markedly in relation to the controls. In contrast, the decrease observed at 42 °C was not statistically significant.

(b) Heat Treatments on Whole Fruits. We analyzed the post-treatment capacity of strawberry to synthesize proteins, just or 48 h after the heat treatment had finished (Figure 4b,c). Immediately after treatment, the incubation at 48 °C reduced protein synthesis compared to the controls, whereas, in that at 42 °C, no significant differences were observed. These results are similar to those described in a) for fruit disks. Nevertheless, after 48 h at 20 °C, the thermally treated fruits partially recovered their capacity to synthesize proteins. Even then, fruits treated at 48 °C showed lower radioactive amino acid incorporation than controls.

Decreased protein synthesis has been reported for thermally treated apple, pear, and tomato by Paull (1990). Our results indicate a similar behavior in strawberry, where protein synthesis was reduced to 10−20% of the control value after treatment at 48 °C for 3 h. This effect was observed both in tissue disks labeled at 48 °C and in whole fruits treated at 48 °C and then labeled at 20 °C. The partial recovery of protein synthesis after 48 h at 20 °C suggests that the influence of heat treatment on strawberry fruit metabolism could be temporary.

In parallel with the above-mentioned quantitative study, we analyzed the composition of synthesized polypeptides by autoradiography. Total protein amount

in all lanes was approximately the same, as observed with the Coomassie-Blue staining (data not shown). Fruit disks treated at 42 °C showed the synthesis of five polypeptides (88, 76.5, 22, 19, and 17 kDa) that were not detected in control fruit disks. The synthesis of a 69 kDa polypeptide increased with respect to controls, as well. It must be pointed out that the 69 and 17 kDa species coincide those referred to previously for Coomassie-Blue staining. After 48 h at 20 °C, both control and treated fruits showed similar bands, and the above-mentioned species (88, 76.5, 22, 19, and 17 kDa) were not detected, so suggesting that the effect of heat treatment on protein synthesis was temporary.

Similar experiments were performed at 48 °C. This heat treatment made a great number of bands to disappear or to weaken their intensity, indicating inhibition of protein synthesis. Again, treated fruits showed five (88, 76.5, 69, 19, and 17 kDa) of the six polypeptides found in 42 °C treatment, the 22 kDa polypeptide being lacking after this treatment. After 48 h at 20 °C both control and treated fruits showed approximately the same profile, suggesting that heat-treated fruits recovered their capacity to synthesize proteins. Regarding the above-mentioned species, we did not detect the 88 kDa polypeptide in treated fruits after 48 h at 20 °C. The 76.5 kDa polypeptide appeared both in control and treated fruits, whereas the synthesis of the 69 kDa polypeptide kept more intense in treated fruits. A persisting difference was that of 19 and 17 kDa polypeptides, which were found only in treated fruits.

The above-described polypeptides can be considered as heat-shock proteins (HSP), as those found previously in several fruits under thermal stress. In this regard, other authors have indicated that heating at 38 °C induced HSP formation in tomato and apple fruits and in cell cultures of pear fruit (Lurie and Klein, 1990; Klein and Lurie, 1991). They have described five HSP with molecular mass values ranging from 15 to 22 kDa, an additional HSP of 70 kDa, and two more above 80 kDa. These species were similar to those found previously in plum and papaya (Tsuji et al., 1984; Paull and Chen, 1990). The precise function of HSP was not totally explained yet, but, at least in yeasts, there is increasing evidence that a 70 kDa HSP can be a cytoplasmic factor involved in the translocation of proteins across membranes (Brodl, 1989).

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Received for review January 13, 1997. Revised manuscript received June 9, 1997. Accepted September 4, 1997.[⊗] This work was supported by grants from CONICET.

JF9700337

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.