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## Quality of heat-treated strawberry fruit during refrigerated storage

Ariel R. Vicente <sup>a</sup>, Gustavo A. Martínez <sup>b</sup>, Pedro M. Civello <sup>b,\*</sup>,  
Alicia R. Chaves <sup>a</sup>

<sup>a</sup> *Centro de Investigación y Desarrollo en Criotecología de Alimentos (CIDCA), CONICET-UNLP, 47 y 116, 1900 La Plata, Argentina*

<sup>b</sup> *Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús (IIB-INTECH), CONICET-UNSAM, Camino de Circunvalación Laguna Km 6, 7130 Chascomús, Argentina*

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### Abstract

Strawberries cv. Selva were heat-treated in an air oven (45 °C, 3 h) and then stored at 0 °C for 0, 7 or 14 days. Afterward, fruits were placed at 20 °C and monitored after 24, 48 or 96 h and the effect of heat treatment on the following parameters was recorded: weight loss, external color, anthocyanin content, firmness, titratable acidity, total and reducing sugars, fruit decay and count of colony forming units (CFUs) for bacteria and molds. Heat-treated fruits showed higher hue angle than controls, indicating the delay of red color development. The treatment diminished fruit lightness ( $L^*$ ), although the effect reverted during holding at 20 or at 0 °C. The application of the treatment caused an initial weight loss close to 2% but afterwards, heat-treated fruits showed lower weight loss rate at 20 °C. Heat-treated fruits had lower acidity than controls, but there was no difference in the content of total sugars between control and treated fruits. Heated fruits were slightly firmer at the end of the treatment, and they softened less than controls after 24 h at 20 °C. Heat-treated fruits remained firmer than controls after 7 days of cold storage, and the relative difference in softening persisted after 48 h at 20 °C. However, no difference in treated and control fruit firmness was observed after 14 days of storage at 0 °C and following 48 h at 20 °C. In the absence of storage, heat-treated fruits showed lower decay at 20 °C than controls. After 7 days at 0 °C followed by 72 h at 20 °C, the percentage of decayed fruits was lower in heat-treated than in control fruits. The treatment decreased the initial bacterial population, but did not modify the amount of mold initially present. After 7 days of cold storage, the CFU number for bacteria were lower in treated than in control fruits. This difference was still significant after 48 h at 20 °C. In the case of molds, heat-treated fruits that were stored for 7 or 14 days at 0 °C and then transferred to 20 °C for 48 h showed lower CFU value than controls. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Postharvest; Heat treatment; Strawberry fruit

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\* Corresponding author. Tel.: + 54-2241-424-049; fax: + 54-2241-424-048.

E-mail address: pmcivello@hotmail.com (P.M. Civello).

## 1. Introduction

The strawberry is a very perishable fruit, with short shelf life mainly due to its soft texture, high softening rate and high sensitivity to fungal attack. Most methods employed to extend shelf life of perishable products are based on using chemical products. Public concern with fungicide residues on the fruit (Peng and Sutton, 1991) and the development of fungicide tolerance in pathogen populations (Hunter et al., 1987) has increased the need to find alternative methods for managing vegetable crops. Therefore, thermal methods are a promising alternative to replace or to reduce the use of chemical treatments during postharvest storage.

The beneficial effect of heat treatments on the storability of different fruits is well documented. The exposure to temperatures higher than 35 °C has caused ripening inhibition in different fruits (Paull, 1990; Lurie, 1998). Heating of mature green tomatoes for 2 or 3 days at 38 °C reversibly inhibited ripening and decreased fruit decay during storage (Lurie and Sabehat, 1997). The effect of heat treatment on fungal decay could be due to a combination of direct inactivation of the pathogen and to the induction of some kind of natural resistance in the fruit (Spotts and Chen, 1987; Klein and Lurie, 1991; Fallik et al., 1995).

Few studies have analyzed the application of heat treatment in the case of soft fruits, and most of them were done on strawberry fruit. García et al. (1995a) found that hot water dips of strawberries cv. Tudla reduced fruit decay, increased the soluble solid content and decreased titratable acidity, hence improving key factors related to sensorial acceptance by the consumer. The application of a different kind of heat treatment (42 or 48 °C for 3 h in an air oven) also delayed fruit ripening and diminished fungal decay of strawberries cv. Selva during storage at 20 °C (Civello et al., 1997). In addition, the treatment at 48 °C for 3 h caused a temporary delay of strawberry fruit softening, a reduction in phenylalanine ammonia lyase (PAL) activity and the diminution of protein synthesis (Civello et al., 1997).

The changes in metabolism induced by the application of heat treatments are temporary, and

after returning to lower temperatures, the fruit tends to recover and continue with normal ripening (Biggs et al., 1988; Klein and Lurie, 1991). Although when heat-treated fruit were stored at room or cold temperatures, some residual effects have been reported (Klein and Lurie, 1990; Conway et al., 1994). Therefore, it is worthwhile to analyze if the combination of heat treatment with other physical treatments could extend these beneficial residual effects.

The present work examines the effect of combining a particular heat treatment (45 °C, 3 h) with refrigerated storage (0, 7 or 14 days at 0 °C) on several parameters related to strawberry fruit quality, and their evolution once the fruit is transferred to shelf conditions.

## 2. Materials and methods

### 2.1. Plant material

Strawberries (*Fragaria* × *ananassa* Duch., cv. Selva) grown in greenhouses were harvested at different ripening stages and classified according to their surface color as 50–75% red or 100% red. Fruits harvested at 100% red were used to measure the effect of heat treatment and refrigerated storage on fruit decay. More immature fruits (50–75% red) were used to analyze the influence of the treatment on thermal profile, firmness, surface color, weight loss, anthocyanin content, pH, titratable acidity, sugar content and the bacteria and mold plate counts.

### 2.2. Heat treatment and storage

Fruits were put in plastic trays covered with PVC film (15 µm thick) and left for 3 h in an air oven set at 45 °C, without air circulation. After treatment, trays were placed at 0 °C for 0, 7 or 14 days and then transferred to 20 °C for 2–4 days. Corresponding controls were not thermally treated but directly brought to 0 °C and then left at 20 °C. Samples were taken at different storage times and were immediately used or frozen in liquid nitrogen and stored at –80 °C until use.

### 2.3. Thermal profile and measure of relative humidity

Changes in the fruit and air temperatures were monitored during application of the heat treatment. A PVC-sheathed copper–constantan thermocouple (1 mm OD) was inserted 10 mm at the equatorial zone of each of four strawberry fruit. Two thermocouples were used to measure the air temperature near the fruit. Data were acquired every 10 s using an acquisition card (Keithley Instruments Inc., Model DAS-TC, Tautow, MA). The experiment was repeated three times.

The change of relative humidity (RH) of the atmosphere surrounding the fruit was followed during the 3 h of heat treatment. A chilled-mirror dew point sensor (Model M4/1111H-SR, General Eastern Instruments, Woburn, MA), connected to a Hygro M1 monitor was used. The sensor was fitted inside a tray containing the fruits and the whole set was covered with PVC film (15  $\mu\text{m}$  thick). Data were acquired every 5 min during heat treatment. RH values were calculated as  $p_s(\text{Tr})/p_s(\text{Ta})$ , Tr being the dew point temperature and Ta the dry bulb value measured both inside the tray, while  $p_s$  is the saturation vapor pressure corresponding to those temperatures. The experiment was repeated three times.

### 2.4. Fruit decay evaluation

Fully red strawberries were heat-treated, stored at 0 °C, then transferred to 20 °C and their external appearance was monitored for 4 days. Thirty berries were used for each storage combination analyzed. To prevent contamination among fruits, each strawberry was put in an individual plastic tray and covered with PVC film. The presence of macroscopic fungal growth was visually evaluated.

### 2.5. Firmness

Fruit firmness was analyzed after each storage time at 0 °C and after 24 and 48 h at 20 °C. The maximum force (N) reached during tissue breakage was measured with an Instron Universal testing machine (Model 1011, Instron Corp., Canton,

MA), fitted with an 8 mm diameter convex probe. The fruits were measured twice on opposite sides of the central zone, and 30 berries per treatment were used for each condition analyzed.

### 2.6. External color

Surface color was evaluated with a colorimeter (Minolta, Model CR-300, Osaka, Japan) by measuring the  $L^*$  parameter and hue angle in six zones of each fruit. Thirty fruits were analyzed for each condition.

### 2.7. Anthocyanins

Frozen fruits were ground by means of a refrigerated mill (Tekmar, model A-10, Cole-Parmer Instrument Company, Chicago, IL) and 1 g of the resultant powder was poured into 10 ml of HCl–methanol (1%, v/v) and held at 0 °C for 10 min. The slurry was centrifuged at  $1500 \times g$  for 10 min at 4 °C, the absorbance at 515 nm of the supernatant was measured and expressed as  $\mu\text{mol}$  of pelargonidin-3-glucoside per kg of fresh weight, using  $E_{\text{molar}} = 3.6 \times 10^6 \text{ M}^{-1} \text{ m}^{-1}$  (Woodward, 1972).

### 2.8. Weight loss

Thirty fruits were used to evaluate weight loss. The same fruits were weighed at the beginning of the experiment, after the heat treatment, during refrigerated storage at 0 °C, and after incubation at 20 °C.

### 2.9. pH and titratable acidity

Frozen fruits were ground in a mill and 10 g of the powder was suspended with H<sub>2</sub>O up to a volume of 100 ml. The pH and acidity of the sample were determined by pH meter and titration with 0.1 M NaOH to pH 8.1 (AOAC, 1980). Titratable acidity was expressed as mmol of H<sup>+</sup> per kg of fresh weight. Two independent samples per condition were analyzed, and each sample was titrated by duplicate.

### 2.10. Reducing and total sugar content

Frozen fruit samples were ground and 1 g of the powder was extracted for 30 min with 10 ml of ethanol at 25 °C. The mixture was centrifuged at  $2300 \times g$  for 10 min and 1 ml of the supernatant was brought to 50 ml with H<sub>2</sub>O. The content of reducing sugars was determined spectrophotometrically at 520 nm using a modification of the Somogyi-Nelson method (Southgate, 1976). For total sugar determination, the samples were first hydrolyzed with 0.1 M HCl for 10 min and then processed as described above. Results were expressed as g of glucose per kg of fresh fruit.

### 2.11. Microbiological assays

Control and heat-treated strawberries were analyzed after 0, 7 or 14 days at 0 °C and 0 or 2 days at 20 °C. Two samples of five fruits each were taken per each storage time analyzed. The five intact strawberries, weighing about 50 g, were stirred altogether in 200 ml of sterile H<sub>2</sub>O for 45 min. From the resulting suspension, two series of dilutions from  $10^{-1}$  to  $10^{-5}$  were prepared and 1 ml of each dilution was seeded in the appropriate medium (Petrifilm™ plates 6400 and 6407, 3M, St. Paul, MN) by triplicate. Plates for aerobic mesophilic bacteria counts were incubated at 30 °C for 3 days; in the case of molds, plates were incubated at 20 °C for 5 days. The plate count was discarded when less than 30 or more than 300 colonies were found. Results were expressed as log of colony forming units (CFUs) per g of fresh fruit. The whole experiment was repeated twice for molds and three times in the case of bacteria. Since the effect of heat treatment had the same general trend in all the experiments, the results from only the second experiment are shown.

### 2.12. Statistical analysis

Experiments were performed according to a factorial design. Data were analyzed by means of ANOVA, and the means were compared by the least significant difference (LSD) test at a significance level of 0.05.

## 3. Results and discussion

### 3.1. Thermal profile and relative humidity during heat treatment

The temperature profile of fruits during heat treatment was analyzed. The air oven temperature was set at 45 °C, and then monitored through the 3 h of treatment. The temperature of fruit flesh increased from an initial value close to 23 °C up to 40 °C in the first hour, then increased to 42 °C during the next 30 min and remained around this value until the end of the treatment (Fig. 1).

RH of the air surrounding the fruits inside the trays rose from 32 to 60% during the first hour of heating and reached 82% at the end of the 3 h of heat treatment (Fig. 1). No visible dehydration was observed.

### 3.2. Weight loss

After treatment, the heat-treated fruits showed a 2% weight loss with respect to the initial weight (Table 1). After 48 h at 20 °C, a clear increase of weight loss was observed in control and treated fruits. As the weight loss rate at 20 °C was proportionally higher in control than in treated fruits, the accumulated weight losses of both groups were not statistically different after 48 or 96 h at 20 °C.

After 7 days at 0 °C, neither treated nor control fruits showed any additional significant weight loss. Again, once the fruits were brought to 20 °C, all fruits showed an increase in weight loss and the accumulated losses did not differ statistically between treated and untreated fruits.

After 14 days of storage, all fruits showed a significant weight loss relative to initial values. In this case, weight loss at 20 °C was similar between control and treated fruits, therefore, after 48 h the accumulated weight loss of heat-treated fruits remained higher than that of controls.

### 3.3. Surface color and anthocyanins

Changes in external color during strawberry fruit ripening were evaluated through the hue

angle and  $L^*$  parameter. Just after heat treatment was finished, no difference in hue angle was observed between treated and control fruits. In all cases, both treated and control fruits showed a decrease in the hue angle through the stay at 20 °C (Table 1). In addition, a significant decrease of hue angle was observed during the storage of treated or control fruits at 0 °C, indicating continued ripening during cold storage. After 0, 7 or 14 days of refrigerated storage and subsequent storage at 20 °C for 48 h, the heat-treated fruits showed higher hue angle than controls. However, this difference was not found after 96 h at 20 °C.

Strawberry fruit lightness was evaluated through the  $L^*$  parameter value. The treatment caused a significant loss of lightness (Table 1), while a steady diminution of  $L^*$  was observed in both control and

treated fruits during storage at 0 °C and during holding at 20 °C. However, in the absence of refrigerated storage, the  $L^*$  value of heat-treated fruits remained higher than in controls after 48 or 96 h at 20 °C. As well, treated fruits showed higher  $L^*$  value after 7 or 14 days of refrigerated storage at 0 °C, but this difference disappeared after 48 or 96 h at 20 °C.

The influence of heat treatment on color development was also followed through the anthocyanin content (Fig. 2). No significant difference in anthocyanin content was found immediately after heat treatment. However, when fruits were held at 20 °C, the treated fruits showed less anthocyanin accumulation than controls. This reduction of anthocyanin synthesis was likely due to the diminution of PAL activity, previously reported in heat-

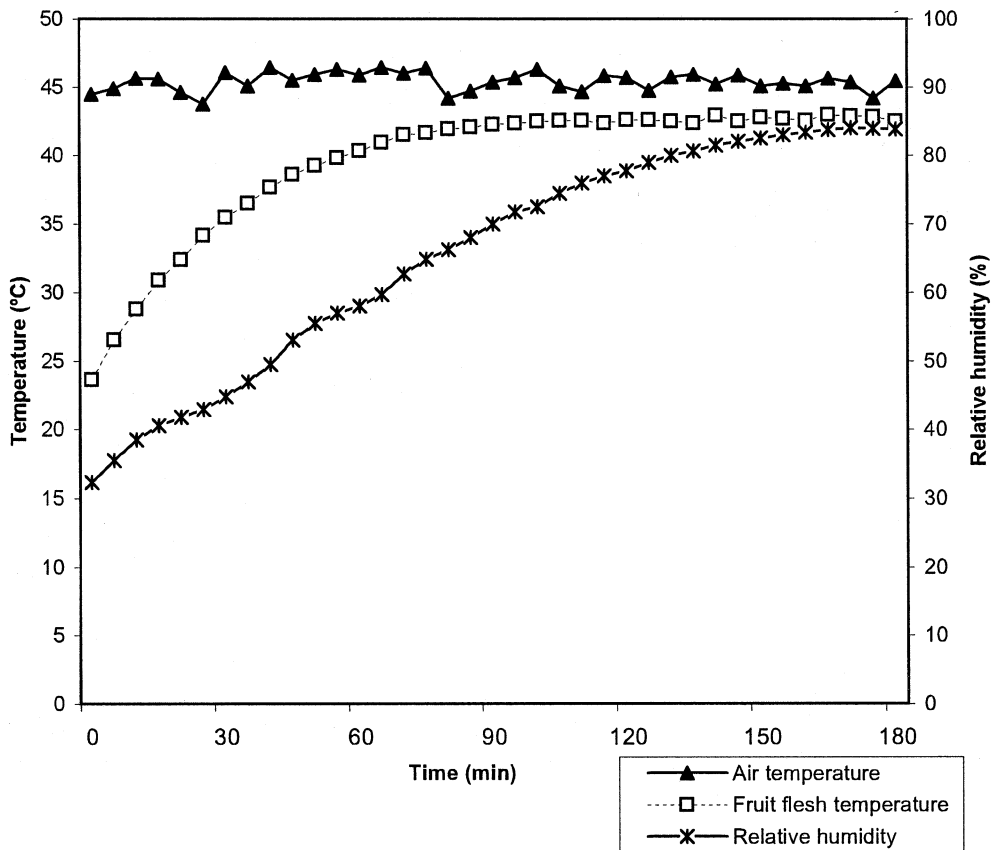


Fig. 1. Thermal profile of strawberry fruits flesh and RH of the atmosphere surrounding the fruits during heat treatment (45 °C, 3 h in air). Temperature data correspond to the average obtained from four fruits.

Table 1

Changes in superficial color and weight loss in control and heat-treated fruits (45 °C, 3 h in air oven) during refrigerated storage at 0 °C and subsequent storage at 20 °C

	Days at 0 °C								
	0			7			14		
	Hours at 20 °C								
	0	48	96	0	48	96	0	48	96
<i>Weight loss (%)</i>									
C	0	2.92	5.85	0.34	3.43	8.47	1.47	4.55	ND
T	2.04*	3.69	6.45	2.18*	3.92	9.54	3.23*	5.66*	ND
<i>Hue</i>									
C	60.71	45.82	42.69	53.78	46.62	40.20	49.77	43.56	39.64
T	60.63	53.60*	44.37	57.98*	48.90*	41.34	50.39	46.37*	40.53
<i>L*</i>									
C	47.65	37.36	33.70	43.26	38.11	30.17	34.41	32.29	28.54
T	45.50*	41.51*	35.78*	44.85*	39.10	30.44	35.58*	32.83	28.41

C, control; T, heat-treated; ND, not determined.

\* The asterisks (\*) indicates the value is statistically different from that corresponding to control ( $P < 0.05$ ).

treated strawberry fruit (Civello et al., 1997). The synthesis of PAL begins at the 'turning' stage and increases through strawberry fruit ripening (Hyodo, 1971; Given et al., 1988a,b). The application of heat treatment interrupts normal protein synthesis (Sachs and Ho, 1986; Brodl, 1989), and PAL accumulation and anthocyanin synthesis are temporarily arrested in heat-treated fruits. After 7 days at 0 °C, the amount of anthocyanins was lower in heat-treated fruits and the difference persisted after 96 h of incubation at 20 °C. Control and treated fruits stored for 14 days at 0 °C showed a similar anthocyanin level, but after the transfer to 20 °C, heat-treated fruits accumulated less anthocyanins than the controls. These experiments indicate that a residual effect of heat treatment on anthocyanin accumulation was still present after 7 or 14 days of refrigerated storage. They also indicate a decrease in the ability of the control fruits to accumulate anthocyanins once stored for 7 days or longer.

### 3.4. Firmness

In strawberry fruit, different types of heat treatments delayed softening (García et al., 1995a;

Civello et al., 1997). Therefore, we decided to analyze the effect of the treatment on texture during storage at 0 °C with subsequent storage at 20 °C. The results of these experiments are shown in Fig. 3. Immediately after the 3 h of treatment at 45 °C, the fruits were slightly firmer than controls. After staying 24 h at 20 °C, heat-treated fruits softened less than controls, but the difference in firmness disappeared after 48 h. Fruits that had been stored for 7 days at 0 °C remained firmer than controls. When fruits were brought to 20 °C, the treated fruits softened slower and remained firmer than controls after 48 h. Instead, after 14 days of storage, no difference was observed between treated and control fruits, neither at the end of the storage nor during subsequent storage at 20 °C.

The firmness of strawberry changes dramatically once the fruit reaches the turning stage (25% red), and its diminution contributes to fruit susceptibility to decay. Therefore, any treatment capable of delaying softening is potentially helpful to extend the postharvest shelf life and to maintain the product quality. The effect of heat treatment in delaying fruit softening has been reported in several fruits, including tomato (Biggs et al.,

1988) and apple (Porrit and Lidster, 1978; Klein and Lurie, 1990). In the latter case, the effect of heat treatment on texture remained after 6 months of storage at 0 °C (Porrit and Lidster, 1978; Klein and Lurie, 1990; Conway et al., 1994).

Delay of softening caused by application of heat treatment on strawberry fruit remained after 7 days at 0 °C (Fig. 3). Heat treatment affects protein metabolism by suspending the synthesis of housekeeping proteins to produce heat-shock proteins (Brodl, 1989). The treatment of strawberry fruit at 42 or 48 °C for 3 h inhibited temporarily the protein synthesis, which was re-established after 24 h at 20 °C (Civello et al., 1997). Therefore, the effect observed in this study is probably due to the temporary suspension of enzyme synthesis involved in cell wall degradation. Once the heat-shock was finished, the synthesis of regular proteins resumed and the effects

of heat treatment on fruit metabolism tended to fade. Storage at 0 °C of heat-treated fruits could delay the recovery of normal fruit metabolism, which includes softening, but only for 7 days. Instead, after 14 days at 0 °C, no difference between treated and control fruit softening was found.

### 3.5. pH and titratable acidity

The pH of homogenates from control or heat-treated fruits was close to 3.6, and no significant pH variation was detected during storage at 0 or 20 °C, for either control or treated fruits (Table 2). However, immediately after treatment the treated fruits showed lower titratable acidity than the controls, and this difference remained after 96 h of incubation at 20 °C (Table 2). The reduction of titratable acidity after application of heat treat-

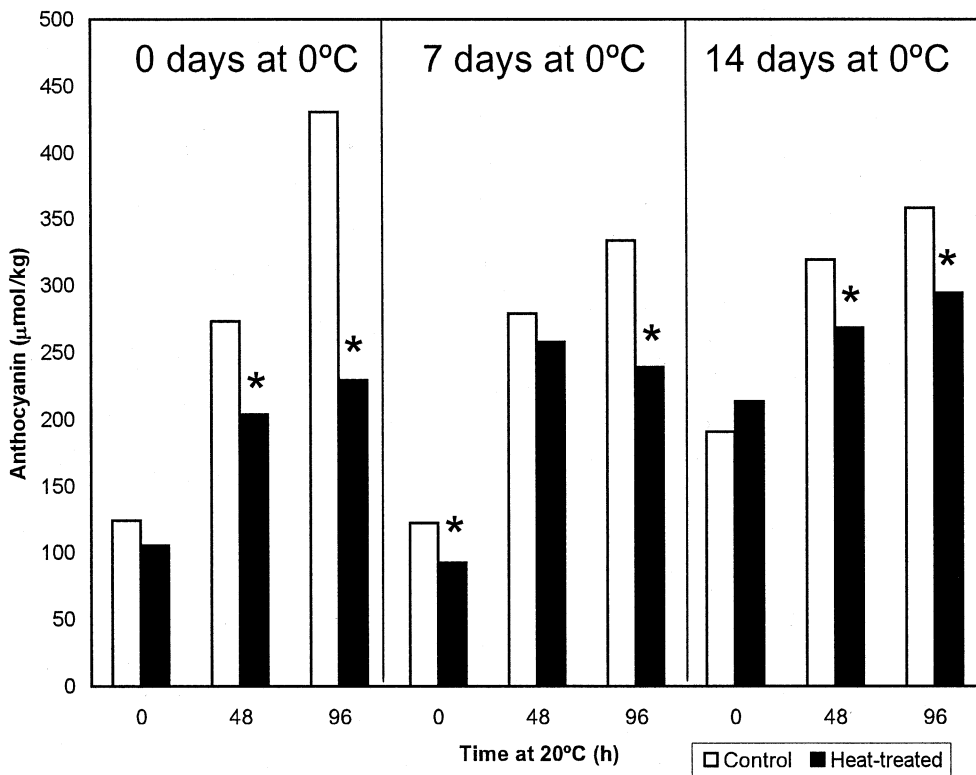


Fig. 2. Changes in anthocyanin content in control and heat-treated fruits (45 °C, 3 h in air oven) during storage at 0 °C and subsequent storage at 20 °C. The asterisk (\*) indicates that the value is statistically different from that corresponding to control ( $P < 0.05$ ).

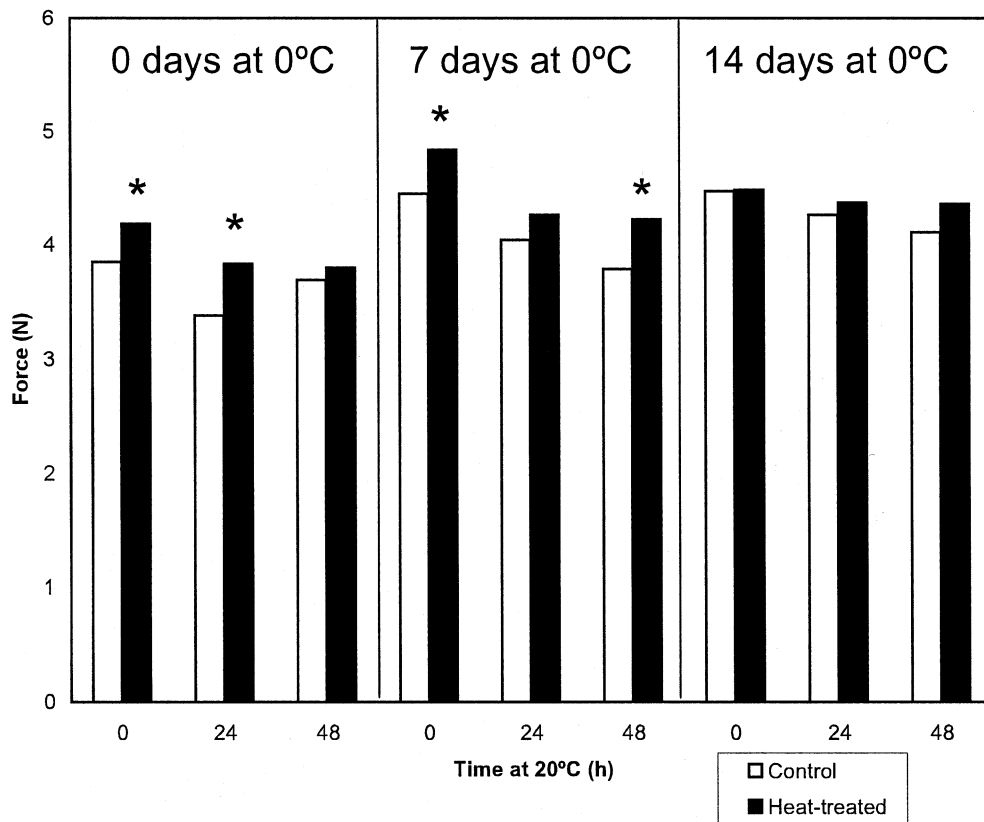


Fig. 3. Changes in firmness in control and heat-treated fruits (45 °C, 3 h in air oven) during storage at 0 °C and subsequent storage at 20 °C. The asterisk (\*) indicates that the value is statistically different from that corresponding to control ( $P < 0.05$ ).

ment has been reported in several fruits, such as apples (Liu, 1978; Klein and Lurie, 1990), nectarines (Lay-Yee and Rose, 1994), grapefruit (Shellie and Mangan, 1994) and tomato (García et al., 1995b). These results also agree with those reported by García et al. (1995a) for strawberries of Tudla variety. A possible explanation is that the application of heat treatment provokes a temporary increase of the respiration rate and that a significant amount of organic acid is then used as substrate in this process (Lurie and Klein, 1990).

After 7 days of refrigerated storage at 0 °C, heat-treated fruits still showed lower titratable acidity than controls, but the difference did not remain after incubation at 20 °C. After 14 days at 0 °C, heat-treated fruits showed higher acidity than controls. Once the fruits were transferred to 20 °C, the acidity of controls increased during the

96 h of incubation while decreased in heat-treated fruits. As will be further discussed, control fruits suffered an important decay after 14 days at 0 °C and 24–48 h at 20 °C that could include some fermentative process responsible for the acidity increase.

### 3.6. Sugar content

The content of total and reducing sugars increased slightly as strawberry fruit ripened during storage, especially at 20 °C (Table 2). The application of heat treatment did not modify the total sugar content, and no significant differences between control and treated fruits were found after storage at 0 °C and further incubation at 20 °C either. Instead, the content of reducing sugars was slightly higher in fruits that had been heat-treated



and then brought to 20 °C for 48 h. Similar results were found in heat-treated fruits after being stored for 14 days at 0 °C and then transferred to 20 °C. Fruits stored for 7 days showed the same trend, but the increase in reducing sugars was not statistically significant.

In ripe strawberry fruit, sugars constitute about 80–90% of soluble solids (Wrolstad and Shallenberger, 1981). In turn, most of the total sugar consists of the reducing sugars fructose and glucose and a small amount of sucrose (Forney and Breen, 1985; Kader, 1991). In strawberry fruit, soluble and wall-bound forms of invertase have been reported (Ranwala et al., 1992). Most of the activity is associated with the soluble invertase fraction along the course of fruit ripening. It is worthwhile to point out that this soluble invertase and one of the cell-wall-bound forms showed high enzymatic activity in the range of temperatures from 40 to 60 °C, the maximum being at 50 °C (Ranwala et al., 1992). Therefore, it is possible to hypothesize that the increase in reducing sugars observed in heat-treated fruits is due to an in-

creased activity of the invertase enzymes at the treatment temperature.

### 3.7. Fruit decay

Heat treatments have been used to diminish postharvest fruit damage caused by pathogens (Klein et al., 1997) in several fruits, including strawberry (Couey and Follstad, 1966; García et al., 1995a; Civello et al., 1997).

The effect of heat treatment followed by refrigerated storage on fruit decay is shown in Fig. 4. In the absence of refrigerated storage and once the strawberries were transferred to 20 °C, the treated fruits showed less fungal lesions than the controls after 48 h. When fruits were stored at 0 °C, neither treated nor control fruits showed any visible rot after 7 or 14 days of storage. In the case of fruits stored 7 days at 0 °C and then transferred to 20 °C, 23% of control fruits showed lesions after the first 24 h at 20 °C, while no statistically significant lesion was observed in the treated fruits (LSD = 20.9%). The percentage

Table 2

Changes in pH, titratable acidity, total sugars and reducing sugars in control and heat-treated fruits (45 °C, 3 h in air oven) during refrigerated storage at 0 °C and subsequent storage at 20 °C

	Days at 0 °C								
	0			7			14		
	Hours at 20 °C								
	0	48	96	0	48	96	0	48	96
<i>pH</i>									
C	3.60	3.53	3.50	3.45	3.50	3.54	3.54	3.56	3.61
T	3.53	3.65	3.53	3.51	3.51	3.59	3.51	3.60	3.69
<i>Titratable acidity (mmol H<sup>+</sup> kg<sup>-1</sup>)</i>									
C	178.6	180.6	203.3	184.7	174.1	191.3	176.0	184.4	203.1
T	168.9*	159.7*	185.2*	179.3*	173.7	185.5	186.6*	180.8	168.7*
<i>Total sugar (g kg<sup>-1</sup>)</i>									
C	48.9	49.5	ND	45.0	53.4	ND	51.2	48.7	ND
T	49.9	48.8	ND	43.0	50.0	ND	51.9	51.2	ND
<i>Reducing sugar (g kg<sup>-1</sup>)</i>									
C	22.3	23.8	ND	24.7	29.7	ND	28.1	29.8	ND
T	22.8	25.8*	ND	25.3	30.8	ND	27.4	31.3*	ND

C, control; T, heat-treated; ND, not determined.

\* The astericks (\*) indicates the value is statistically different from that corresponding to control ( $P < 0.05$ ).

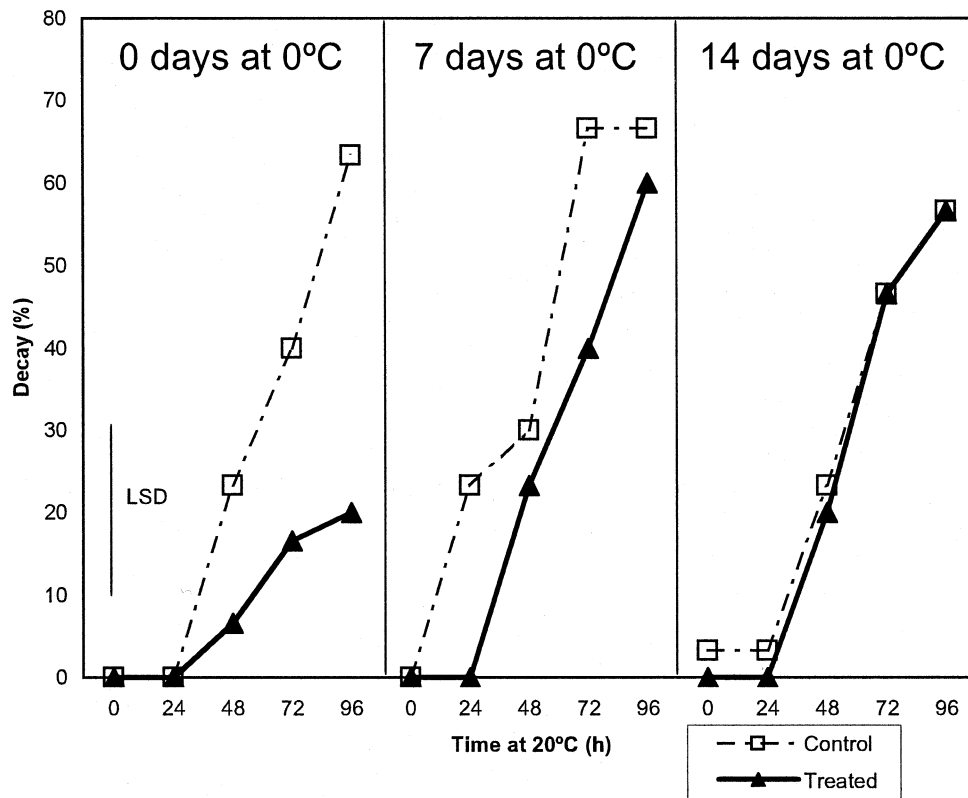


Fig. 4. Development of decay in control and heat-treated fruits (45 °C, 3 h in air oven) during storage at 0 °C and subsequent storage at 20 °C. Each datum corresponds to the percentage of fruits showing fungal attack from a group of thirty fruits. LSD, least significant difference.

of damaged fruits increased during the remaining time at 20 °C in both control and treated populations. Although the percentage of decay was always lower in heat-treated fruits, the difference was statistically significant only in the case of 24 h. Therefore, the data support the idea that heat treatment has a protective effect against fungal attack, and that this effect is even present after 7 days of refrigerated storage. In the case of 14 days of storage at 0 °C, a similar proportion of infected fruits was found in treated or control fruits. Nevertheless, the severity and number of visible lesions were still lower in heat-treated fruits (data not shown).

As growth of molds and bacteria is one of the main causes of postharvest losses in horticultural crops, counts of both types of microorganisms were performed to analyze the effect of heat treat-

ment on fruit decay. Immediately after heat treatment, the number of CFUs of mesophilic bacteria per g of fresh weight was lower in heat-treated than in control fruits (Fig. 5). This reduction of bacterial counts could be due to a direct effect of heat treatment on bacterial viability. After 48 h at 20 °C, no significant difference was found. When fruits were stored at 0 °C for 7 days, the fruits that had been heat-treated showed a lower CFU number, and in this case the difference persisted after 48 h of incubation at 20 °C. No significant difference was found in fruits stored 14 days at 0 °C.

The mold counts shown indicate that the log of CFU per g was not significantly affected immediately after heat treatment or after 48 h at 20 °C (Fig. 6), suggesting that the thermal shock applied would not reduce directly the number of molds

present in the samples. After 7 days of storage, the log of CFU per g value increased in both control and treated fruits, but no difference was observed. However, when stored fruits were brought to 20 °C, the heat-treated fruits showed a significant reduction in the log of CFU per g number. Similarly, lower log of CFU per g was found in treated fruits after 14 days of storage and 48 h at 20 °C. It is worthwhile to point out that the mold CFU number in Fig. 6 correlates only partially with actual fruit decay (Fig. 4). An increase in the decay at 20 °C was generally associated with an increase in CFU number. However, fruits stored for 14 days at 0 °C showed similar decay but lower CFU number than fruits stored for 7 days. A possible explanation is that viable fungal spores decrease during storage at 0 °C, but fruits become more sensitive to fungal

invasion because of its more advanced ripening stage and softening. Another possibility is that stress caused by heat treatment created a response that protects the fruits against a fungal attack. Sabehat et al. (1998) reported that the exposure of an organism to thermal stress could raise some kind of protection against other type of stress. Therefore, the significant reduction of mold CFU per g observed in treated fruits after refrigerated storage and incubation could be due to a physiological response of the fruit tissue. Lurie et al. (1997) described the production of defense-associated enzymes in heat-treated tomatoes. The presence of antifungal substances, including phytoalexins and elicitors of defense mechanism activation, has been described in tissues exposed to several abiotic stresses (Darvill and Alberheim, 1984).

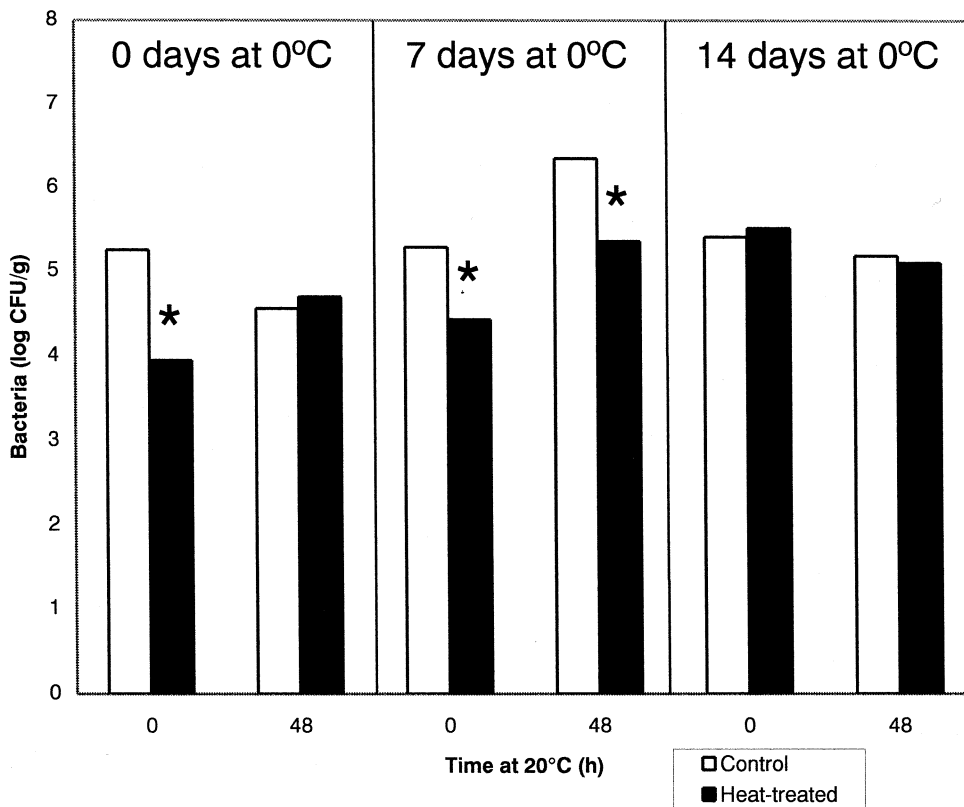


Fig. 5. CFUs (log CFU per g) for mesophilic bacteria in control and heat-treated fruits (45 °C, 3 h in air oven) during storage at 0 °C and subsequent storage at 20 °C. The asterisk (\*) indicates that the value is statistically different from that corresponding to control ( $P < 0.05$ ).

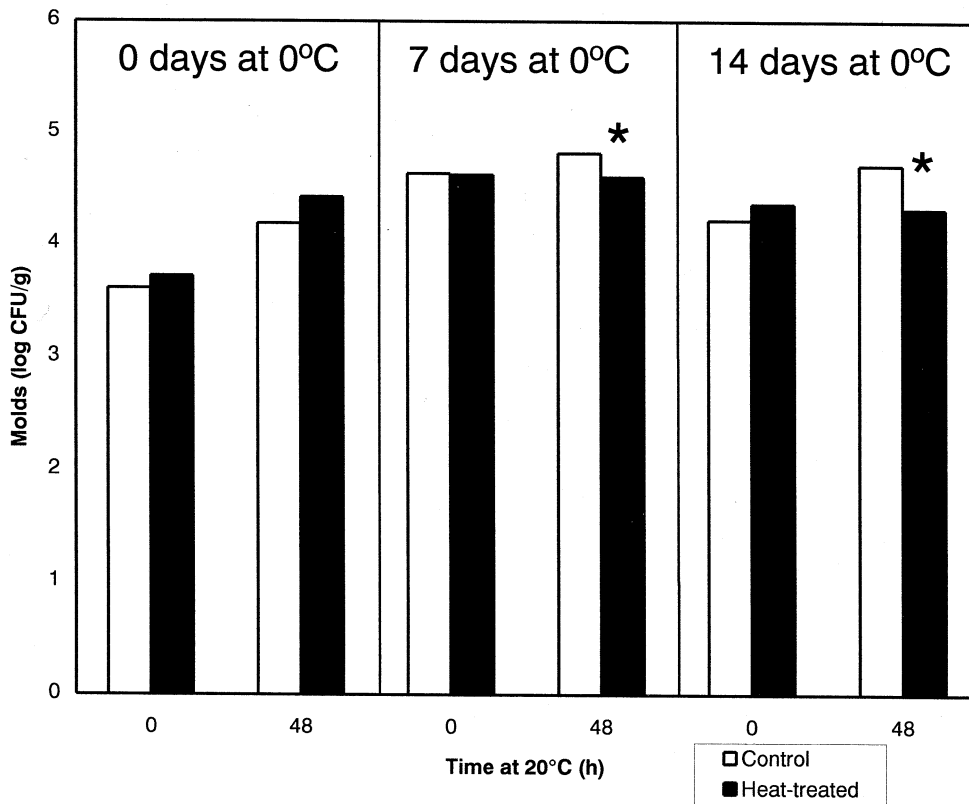


Fig. 6. CFUs (log CFU per g) for molds in control and heat-treated fruits (45 °C, 3 h in air oven) during storage at 0 °C and subsequent storage at 20 °C. The asterisk (\*) indicates that the value is statistically different from that corresponding to control ( $P < 0.05$ ).

#### 4. Conclusions

According to all the parameters analyzed in this study, treatment of strawberry fruit at 45 °C for 3 h in air delayed ripening and fruit decay once the fruits were brought to 20 °C. When heat-treated fruits were stored at 0 °C, the residual effect of the heat treatment was uneven on the different parameters analyzed. The most persistent effects were the delay in anthocyanin accumulation and the diminution of viable fungi (log CFU per g), both of which remained after 14 days of storage and 48 h at 20 °C. The diminution of viable bacteria and number of infected fruits and the delay in softening were clearly observed after 7 days of storage and 48 h at 20 °C, but the differences between treated and control fruits disappeared

after 14 days of cold storage. The results obtained in this first work on applying a combination of heat treatment and cold storage to strawberries are encouraging. Heat treatments combined with refrigerated storage could be a methodology to maintain fruit quality and to diminish decay. More research is needed in this area to improve the treatment conditions and to understand the effect of heat treatments on metabolism of highly perishable fruits, such as strawberries.

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