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Influence of self-produced CO_2 on postharvest life of heat-treated strawberries

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Abstract

Strawberries cv. Selva (75 or 90% superficial red color) were packaged with films having different permeability properties, heat-treated in an air oven (45 °C, 3 h), stored at 0 °C for 0, 7 or 14 days and then transferred to 20 °C for 2 days. The percentage of CO_2 in the package atmosphere and the effect of heat treatment on the following parameters were recorded: weight loss, external color, lightness, anthocyanin content, firmness, titratable acidity, pH, total sugar, total phenol and fruit decay. The application of heat treatment alone reduced fungal decay and delayed red color development and fruit softening. When the treatment was performed on fruit in films that allowed retention of part of CO_2 produced during heating, the delay of fruit softening and color development and the reduction of decay were enhanced. The benefit for 90% red fruit was minimal and decay development was only delayed by 1 day. The results of this work indicate that the well-known benefits of heat treatment could be considerably improved by performing the treatment in the presence of low permeability films, with the aim of retaining the CO_2 produced by the temporary increase in fruit respiration during the heating.

Keywords: Film; Postharvest; Heat treatment; Carbon dioxide; Strawberry

1. Introduction

The risks of improper use of chemicals in postharvest technology have been recognized and consumers are looking for safer products. The demand of organic products is growing very fast, and there is a renewed interest in the development of physical methods that could complement or replace the application of chemicals.

Among physical technologies, the use of refrigeration and temperature management is widely used to reduce spoilage and extend product post-

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harvest life (Mitchell, 1992). In the case of a very perishable fruit like strawberry, the use of refrigerated storage is recommended (Kader, 1991).

Heat treatments have been successfully used for different products e.g. apple, tomato, and mango (Klein and Lurie, 1991; Conway et al., 1994; Paull, 1990; Lurie, 1998; Lurie and Klein, 1990). Heat treatments with hot water or air have been used for different commodities to reduce the incidence of postharvest diseases (Lurie, 1998). Fallik et al. (1995) described the use of heat treatments on apples to diminish decay due to *Penicillum expansum*. In the case of strawberries, the benefit of using heat treatment has been reported (Couey and Follstad, 1966). García et al. (1995) found that hot water dips of strawberry cv. Tudla reduced decay and improved key factors related to sensorial acceptance by the consumer. The application of a different type of heat treatment (42 or 48 °C for 3 h, in an air oven) also delayed fruit ripening and diminished fungal decay of strawberry cv. Selva during storage at 20 °C (Civello et al., 1997). Vicente et al. (2002) reported that heat treatments in combination with refrigerated storage maintained fruit quality and decreased decay in strawberry cv. Selva.

Beneficial effects of CO_2 application on postharvest life of strawberries have been reported (Gil et al., 1997; Holcroft and Kader, 1999; El-Kazzaz et al., 1983). Atmospheres containing 10–15% of CO_2 reduced *Botrytis cinerea* growth and extended strawberries storage (Agar et al., 1990). Modified atmosphere packaging (MAP) can also extend the shelf life of many intact horticultural products included strawberry (Kader et al., 1989). Bai et al. (2001) reported that an additional benefit may be attained by actively flushing the package with the desired gas. The combination of heat treatment with controlled atmosphere has been successfully applied to extend the storage of 'Fuyu' persimmons (Burmeister et al., 1997).

The objective of the present work was to analyze the effect of combined physical treatments (CO_2 and heating) on strawberries preservation, using the increase in fruit respiration produced during the heating as CO_2 source. Since the fruit response to CO_2 and heat treatment could be influenced by the ripening stage, two different stages (75 and 90% red) were analyzed.

2. Materials and methods

2.1. Assay I

Strawberries (*Fragaria x ananassa* Duch., cv. Selva) grown in greenhouses were harvested when they had 75% red color. Fungicides were applied during production according to regular strawberry culture techniques. Ten fruit weighing about 100 g were put in plastic trays and sealed with PVC (O_2 transmission rate 5.72×10^{-17} mol s^{-1} mm^{-2} Pa^{-1}, CO_2 transmission rate 2.47×10^{-16} mol s^{-1} mm^{-2} Pa^{-1} and H_2O vapor transmission rate 2.04×10^{-19} mol s^{-1} mm^{-2} Pa^{-1}) or PD-961EZ film (Cryovac®, W.R. Grace & Co. Conn, USA; O_2 transmission rate 3.57×10^{-17} mol s^{-1} mm^{-2} Pa^{-1}, CO_2 transmission rate 1.07×10^{-16} mol s^{-1} mm^{-2} Pa^{-1} and H_2O vapor transmission rate 6.80×10^{-20}–8.70×10^{-20} mol s^{-1} mm^{-2} Pa^{-1}) and left for 3 h in an air oven set at 45 °C. After treatment, the trays were placed at 0 °C for 0, 7 or 14 days and then transferred to 20 °C for 2 or 5 days. Corresponding controls were not thermally treated and directly brought to 0 °C and then left at 20 °C.

Three samples were taken at different storage times and were immediately used or frozen in liquid nitrogen and stored at −80 °C until assayed for anthocyanin content, sugars, phenolics, pH and titratable acidity.

2.2. Assay II

Fruit were harvested when the surface color was 90% red, put in plastic trays in PY8 (1544 1.7 mm diameter perforations m^{-2}, Cryovac®) or PD-961EZ film and left for 3 h in an air oven at 45 °C. After treatment, the trays were placed at 0 °C for 0, 7 or 14 days and then transferred to 20 °C for 4 days. The controls were placed directly at 0 °C. Samples were taken at different storage times and they were immediately used or frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Gas measurement

2.3.1. Atmosphere composition inside the packages

Package headspace gas samples were withdrawn with a 1-ml syringe from the trays and the content of CO_2 and O_2 was determined using a gas chromatograph (Varian, CX 3400, CA, USA) equipped with a capillary column (Alltech CTR I) and a thermal conductivity detector. Temperatures in the injector, column and detector were set at 120, 30 and 120 °C, respectively. Helium was used as carrier. The percentage of CO_2 inside the trays was calculated. A standard solution containing 5% CO_2, 5% O_2, and 90% N_2 was used as standard for calibrating. Measurements were done on both assays (75 and 90% red fruit).

2.3.2. Fruit respiration rate

Carbon dioxide accumulation during heat treatment and storage was measured by placing about 100 g of whole fruit into 500-ml jars and closing them. Samples were withdrawn with a 1-ml syringe through a septum fitted in the jar lid. Gas analysis was performed by using gas chromatography as described above. Measurements were performed by triplicate.

2.4. Fruit decay evaluation

The presence of fungal growth was visually evaluated daily for 5 (75% red) or 4 (90% red) days at 20 °C after 0, 7 or 14 days at 0 °C. Thirty berries were used for each storage duration and combination analyzed and the percentage of fruit showing fungal lesions was calculated.

2.5. Weight loss and relative humidity measurements

Thirty berries for each treatment and film combination analyzed were used. The same fruit were weighed at the beginning of the experiment, after the heat treatment, during storage at 0 °C for 0, 7 and 14 days and subsequent stay at 20 °C for 2 days. Results were expressed as percentage of weight loss relative to the initial value.

The change in 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, USA), connected to a Hygro M1 monitor was used. The sensor was fitted inside a tray containing the fruit and the whole set was in PVC film (15 μm thick). Data were acquired every 5 min during heat treatment. RH values were calculated as ps(Tr)/ps(Ta), Tr being the dew point temperature and Ta the dry bulb value measured both inside the tray, while ps is the saturation vapor pressure corresponding to those temperatures.

2.6. Superficial color

Surface color was evaluated with a colorimeter (Minolta, Model CR-300, Tokyo, Japan) by measuring the L* and hue parameters in six zones of each fruit. Thirty fruit were assayed after 0, 7 and 14 days at 0 °C plus 1 and 2 days at 20 °C for each treatment combination analyzed. Measurements were done on both assays (75 and 90% red fruit).

2.7. Anthocyanins

One gram of frozen tissue was ground by means of a refrigerated Janke & Kunke mill, Model A-10 (Janke & Kunkel GmbH & Co. KG-IKA-Labortechnik, Staufen, Germany) and the resultant powder was added to 10 ml of methanol containing HCl (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 supernatant was saved and its absorbance at 515 nm was measured. The amount of anthocyanins was calculated using the extinction coefficient (ε) equal to 3.6×10^6 1 mol^{-1} m^{-1} (Woodward, 1972). Anthocyanin content was expressed as micromoles of pelargonidin-3-glucoside per kg of fresh fruit. Measurements were done on both assays (75 and 90% red fruit).

2.8. Firmness

The firmness was measured after 0, 7 and 14 days at 0 °C plus 1 and 2 days at 20 °C using a texture analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY) fitted with a

flat probe. Each fruit was compressed 2 mm at a rate of 0.5 mm s^{-1} and the maximum force developed during the test was recorded. Each fruit was measured twice on opposite sides of its equatorial zone and 30 berries at each condition were assayed. Measurements were done on both assays (75 and 90% red fruit).

2.9. pH and titratable acidity

Fruit acidity and pH were measured after 0, 7 and 14 days at 0 °C plus 1 and 2 days at 20 °C Frozen fruit were ground in a refrigerated mill and 10 g of the powder was suspended with water up to a volume of 100 ml. The pH of the homogenate was measured and the acidity was determined by titration with 0.01 M NaOH up to pH 8.1 (AOAC, 1980). Titratable acidity was expressed as millimoles of H^+ kg^{-1} of fresh fruit. Two independent samples per condition were analyzed, and each sample was titrated by duplicate. Measurements were done on both assays (75 and 90% red fruit).

2.10. Sugar content

The content of sugar was measured after 0, 7 and 14 days at 0 °C plus 1 and 2 days at 20 °C. Frozen fruit samples of 10 g were ground in a refrigerated mill and 1 g of the powder obtained 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 3 ml of the supernatant were brought to 100 ml with water. Total sugars were determined spectrophotometrically at 520 nm by using a modification of the Somogyi–Nelson method (Southgate et al., 1976). Measurements were performed in triplicate and the results were expressed as grams of sugar per kg of fresh fruit. Measurements were done on both assays (75 and 90% red fruit).

2.11. Total phenolic compounds

Frozen samples were measured after 0, 7 and 14 days at 0 °C plus 1 and 2 days at 20 °C. One gram of fruit was ground in 6 ml of ethanol and then the mixture was centrifuged at $9000 \times g$ for 10 min at 4 °C. Three milliliters of the resultant supernatant were brought to 100 ml with water and these solutions were used to determine total phenolic compounds. Two hundred microlitres of extract were added to 1.11 ml of water and 200 μl of 1 N Folin–Ciocalteau reagent. After 3 min at 25 °C, 1.5 ml of saturated solution of Na_2CO_3 were added, and the reaction mixture was incubated for 1 h at the same temperature. The absorbance was measured at 760 nm. Total phenolic content was calculated by using phenol as standard. Measurements were performed by triplicate and results were expressed as g of phenol per kg of fruit. Measurements were done on both assays (75 and 90% red fruit).

2.12. Statistical analysis

Experiments were performed according to a factorial design. Data were analyzed by means of ANOVA, being time at 0 °C, time at 20 °C, type of film used and treatments the factors. The main effects and the interactions were analyzed and the means were compared by the LSD test at a significance level of 0.05.

3. Results and discussion

3.1. Assay I (fruit with 75% red color)

3.1.1. Atmosphere composition

After heat treatment, the CO_2 concentration inside the trays in PVC or PD961EZ was close to 5%, while the CO_2 level in control trays was 0.03% (Fig. 1A).

The accumulation of CO_2 in the heat-treated trays was mainly due to the increase of fruit respiration during heating. The respiration rate of strawberries changed from an initial level of 4.0×10^{-7}–2.4×10^{-6} mol kg^{-1} s^{-1} at the end of the treatment. Lurie and Klein (1990) found that the respiration rate of apples remained at 38 °C was enhanced, and that initial levels were recovered after the treatment. After 1 day at 20 °C, the CO_2 concentration increased up to 6.5% in heat-treated fruit in PD961EZ, compared with 5.1% in the respective control. The CO_2 concentration maintained high after 2 days at 20 °C in heat-

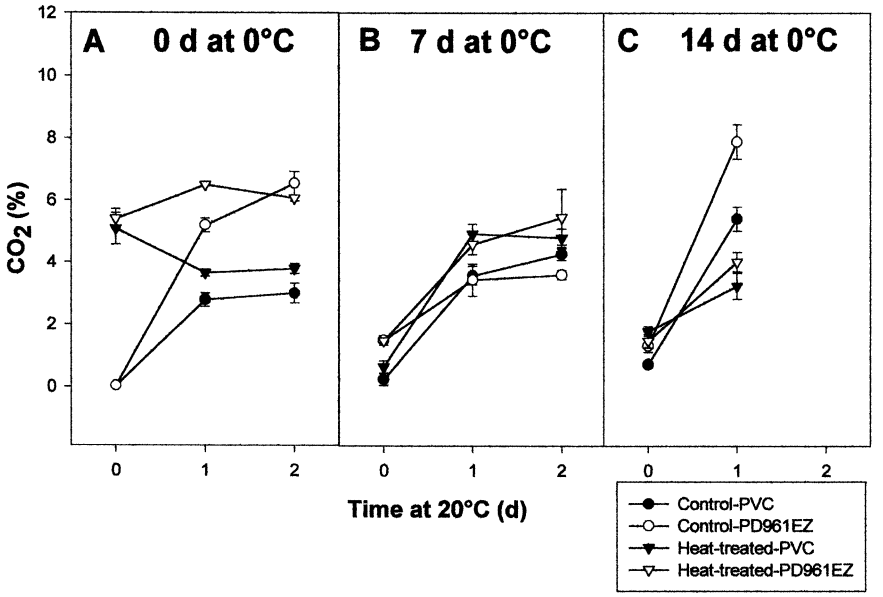

Fig. 1. Change in CO_2 percentage inside trays containing 100 g control and heat-treated strawberries (75% red at harvest), sealed in PVC or PD961EZ film, during storage at 0 °C and subsequent storage at 20 °C. Vertical bars represent S.E.

treated fruit in PD961EZ though a slight diminution was observed. The PD961EZ control accumulated CO_2 steadily during the storage at 20 °C and after 2 days the CO_2 concentration was nearly the same as that in heat-treated fruit ($P < 0.05$).

Immediately after treatment, fruit in PVC accumulated similar CO_2 levels to that found in PD961EZ-covered trays. After 1 day at 20 °C, the CO_2 concentration decreased to 3.6% and did not change further after 2 days. The PVC-control trays accumulated similar CO_2 concentrations to heat-treated fruit after 1 and 2 days at 20 °C ($P < 0.05$).

After 7 days of refrigerated storage (Fig. 1B), the concentration of CO_2 was near 1% in all control and heat-treated fruit in PVC or PD961EZ. During subsequent storage at 20 °C, the CO_2 level increased more in treated than in control fruit, the difference being higher for fruit in PD961EZ after 2 days. A different behavior was observed after 14 days of refrigerated storage (Fig. 1C), the CO_2 level remaining around 1% in all the trays. However, after 1 day at 20 °C, the control fruit accumulated more CO_2 than the heat-treated fruit. This trend was found with both PVC and PD961EZ films though the difference in CO_2 accumulation was greater in the case of the latter. As control fruit suffered more damage and fungal attack during storage, the CO_2 accumulation found in control trays was likely due to the enhanced production of CO_2 from fruit respiration in response to damage and the CO_2 from fungal metabolism. The O_2 levels inside the trays decreased as CO_2 increased, the sum of CO_2 plus O_2 levels being near 21% (data not shown).

3.1.2. Fruit decay

In the absence of cold storage, the control fruit in PVC began to show fungal decay after 2 days at 20 °C while the control fruit in PD961EZ began to show decay 1 day later (Fig. 2A). The differences in decay between the films were accentuated during storage at 20 °C and after 5 days, 67% of control fruit in PVC were decayed while only 27% of control fruit in PD961EZ showed fungal attack. In the case of both films used in this work, the heat-treated fruit showed lower decay percentage than the corresponding control. In addition, the best results were observed in heat-treated fruit wrapped with PD961EZ film; after 5 days at

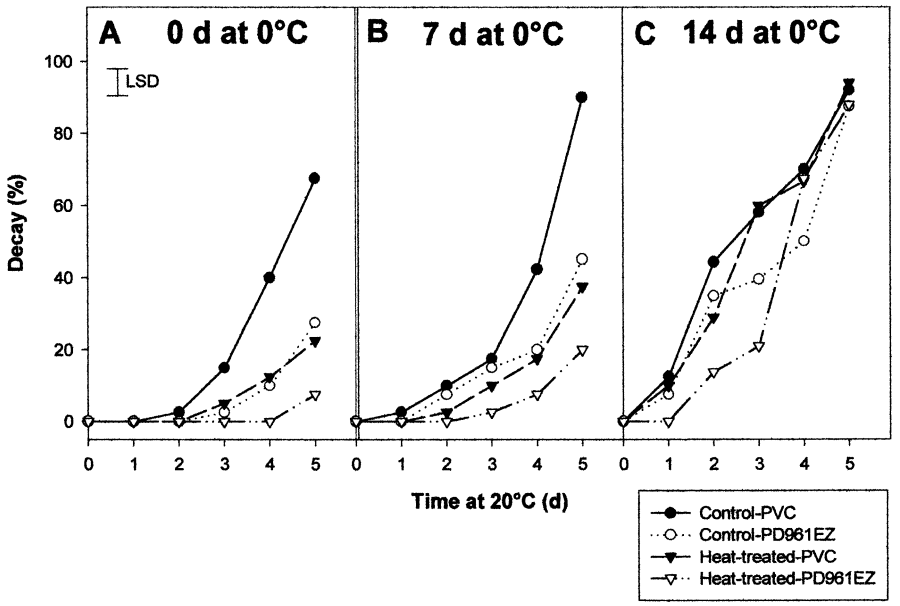

Fig. 2. Decay percentage of control and heat-treated fruit (75% red at harvest), sealed in PVC or PD961EZ film, during storage at 0 °C and subsequent storage at 20 °C. Error bar indicate the least significant difference (LSD) at $P = 0.05$.

20 °C, only 7% of those fruit showed decay while 27% of the controls were decayed.

After 7 days at 0 °C, no fungal attack was observed in any control or heat-treated fruit (Fig. 2B). Once the fruit were transferred to 20 °C, fungal growth was observed in all the conditions analyzed within 3 days. Regarding the percentage of decay, the trends were similar to those found in absence of cold storage. After 5 days at 20 °C, 90% of PVC-control fruit and 45% of PD961EZ-control fruit showed fungal decay while 37% of PVC-treated fruit and only 20% of PD961EZ-treated fruit showed fungal attack. Once more, the best results were obtained when the heat treatment was performed in the presence of PD961EZ, the film that allowed a greater retention of CO_2.

Just after storage, fruit maintained for 14 days at 0 °C did not show external signs of fungal attack (Fig. 2C). However, decay was observed once the fruit were transferred to 20 °C. No difference between control and heat-treated fruit in PVC was found ($P < 0.05$). In the case of fruit in PD961EZ film, heat-treated fruit showed less decay than controls ($P < 0.05$) during the first 3 days of incubation at 20 °C, but no difference was found thereafter.

The reduction of decay described above resulted from the combined effect of heat treatment and an atmosphere enriched in CO_2. The reduction of strawberries decay by heat treatment application has been reported (García et al., 1995; Civello et al., 1997). In turn, the presence of elevated levels of CO_2 has been reported to reduce fungal decay. Carbon dioxide has been shown to retard the germination of fungal spores (Agar et al., 1990). Exposure to CO_2 levels between 5 and 20% has a fungistatic effect on strawberry pathogens (Couey and Follstad, 1966; El-Kazzaz et al., 1983).

3.1.3. *Weight loss*

During the heat treatment, the relative humidity inside the bags increased rapidly and at the end of the treatment reached 84 or 80% for trays in PD961EZ or PVC, respectively (data not shown). After treatment, the fruit in PVC lost 2.9% of their initial weight while the fruit in PD961EZ lost 2.3% (Table 1). Once the trays were transferred to 20 °C, a rapid weight loss was observed, especially

Table 1
Change in weight loss, hue, lightness (L*), titratable acidity, total sugar and total phenols in control and heat-treated strawberries harvested when 75% red, sealed in film PD961EZ or PVC film packages, during refrigerated storage and subsequent storage at 20 °C

		0 days at 0 °C		7 days at 0 °C		14 days at 0 °C	
Days at 20 °C		0	2	0	2	0	2
Weight loss (%)	C_{PVC}	0	6.3	2.2	5.0	2.6	8.9
	C_{PD-961}	0	3.5	1.7	2.2	1.6	2.7
	T_{PVC}	2.9	6.5	3.3	5.2	3.2	9.2
	T_{PD-961}	2.3	4.6	2.2	2.4	2.2	3.2
	LSD	0.8					
Hue	C_{PVC}	59.1	42.9	53.4	45.0	51.5	42.4
	C_{PD-961}	60.5	43.1	57.8	44.4	53.7	46.4
	T_{PVC}	57.0	47.0	56.3	48.1	50.2	47.8
	T_{PD-961}	56.6	48.8	52.2	50.4	54.4	46.7
	LSD	3.6					
L*	C_{PVC}	35.7	29.7	33.4	31.3	33.3	30.0
	C_{PD-961}	36.4	30.5	35.2	31.3	33.3	31.8
	T_{PVC}	35.0	32.5	35.4	33.6	33.0	32.5
	T_{PD-961}	34.4	34.3	35.1	34.0	33.7	32.5
	LSD	1.7					
Titratable Acidity (mmol kg^{-1})	C_{PVC}	124.1	148.7	128.3	141.6	139.0	143.9
	C_{PD-961}	130.0	124.7	130.6	144.8	133.9	135.9
	T_{PVC}	132.3	134.0	133.4	135.2	140.9	126.3
	T_{PD-961}	125.8	129.5	126.4	127.5	120.3	129.0
	LSD	9.2					
Total sugar (g kg^{-1})	C_{PVC}	37.5	27.2	36.0	28.7	28.7	24.0
	C_{PD-961}	37.5	27.8	33.0	30.1	28.7	25.2
	T_{PVC}	35.9	25.7	32.2	26.8	28.0	22.9
	T_{PD-961}	35.5	32.6	33.0	27.3	30.0	22.9
	LSD	4.7					
Total phenols (g kg^{-1})	C_{PVC}	1.7	1.6	1.7	2.5	2.0	2.3
	C_{PD-961}	1.7	1.7	1.6	1.9	1.9	1.9
	T_{PVC}	1.8	1.6	1.7	1.8	2.1	1.8
	T_{PD-961}	1.7	1.7	1.8	1.8	1.7	1.9
	LSD	0.4					

C, control; T, heat-treated. In each case, the LSD at $P = 0.05$ is indicated.

in those fruit in PVC. After 2 days, heat-treated fruit in PVC lost 6.5% of their initial weight while the corresponding control lost 6.3%. Lower weight losses were found for fruit in PD961EZ: 4.6 and 3.5% for heat-treated and control fruit, respectively. Similar trends were found after 7 or 14 days of refrigerated storage and subsequent storage at 20 °C for 2 days.

3.1.4. Surface color and anthocyanins

Immediately after the heat treatment, treated fruit in PD961EZ but not PVC had lower hue values than the corresponding controls (Table 1). After 2 days at 20 °C, all fruit showed a superficial reddening along with a reduction of the hue angle. However, heat-treated fruit in PD961EZ or PVC showed significantly greater hue than the corresponding controls ($P < 0.05$), according to the well known effect of heat treatment in delaying strawberries color development (Civello et al., 1997). No significant difference ($P < 0.05$) between heat-treated fruit in either film was found. When superficial color was measured after 7 or 14 days of refrigerated storage, lower hue values were found in all the fruit, indicating that the pigment accumulation continued at 0 °C. After 7 days at 0 °C followed by 2 days at 20 °C heat-treated fruit showed a delayed color development, but

after 14 days at 0 °C plus 2 days at 20 °C color development was delayed only in PVC packed fruit.

Heat treated fruit in PD961EZ presented lower lightness than the controls just after treatment (Table 1). However, after 2 days at 20 °C, the heat-treated fruit in both PVC and PD961EZ had a higher L* value than the controls; in turn, the lightness loss was less in the case of fruit packed in PD961EZ. After 7 days of refrigerated storage at 0 °C and 2 at 20 °C, the decrease of L* value was less evident in heat-treated fruit. After 14 days at 0 °C and 2 days at 20 °C the L* value continued decreasing, and only those heat-treated fruit in PVC film were still higher than the corresponding control ($P < 0.05$).

After heat treatment, there were no significant differences in anthocyanin content among all the conditions analyzed (Fig. 3A). During storage, heat-treated fruit developed lower anthocyanin content than the respective controls. These levels were different for both films; heat-treated fruit in PD961EZ accumulated less anthocyanin than those in PVC film ($P < 0.05$).

Civello et al. (1997) found that heat treatment reduces the phenylalanine ammonia-lyase (PAL) activity, while Holcroft and Kader (1999) reported that anthocyanin synthesis could be down regulated by CO_2. Therefore, the results obtained in this work could be explained through the combined inhibitory effect of heat treatment and initial accumulation of CO_2 on anthocyanin synthesis.

3.1.5. Firmness

When the treatment was finished and after 2 days at 20 °C, there were not significant differences among treatments (Fig. 4). After 7 days at 0 °C there were no significant differences among treatments either, but heat-treated fruit in PD961EZ film remained the firmest after storage at 20 °C for 2 days ($P < 0.05$).

After 14 days at 0 °C and subsequent storage at 20 °C for 2 days, heat-treated fruit in either film remained firmer than the corresponding controls. Heat treatment has been shown to delay some metabolic processes associated with fruit ripening (Lurie, 1998). Cell wall disassembly is a key ripening-associated event that determines the extent of fruit softening and contributes to the ultimate deterioration of the fruit (Fischer and Bennett, 1991). The delay of fruit softening, in the case of heat-treated fruit especially in those in PD961EZ film, could enhance host resistance to pathogen invasion and then contribute to the

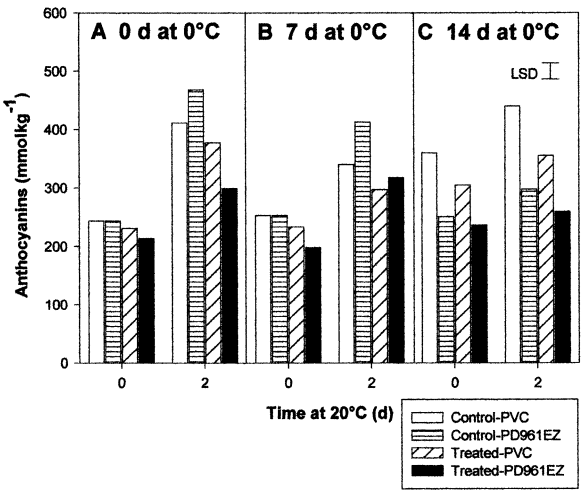

Fig. 3. Changes in anthocyanin content in control and heat-treated fruit harvested when 75% red, sealed in PVC or PD961EZ film, during storage at 0 °C and subsequent storage at 20 °C. Error bar indicate the LSD at $P = 0.05$.

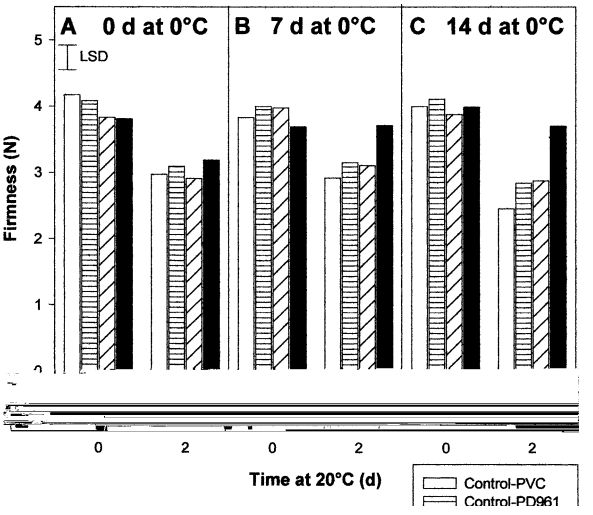

Fig. 4. Changes in fruit firmness in control and heat-treated fruit harvested when 75% red, packed in PVC or PD961EZ film, during storage at 0 °C and subsequent storage at 20 °C. Error bar indicate the LSD at $P = 0.05$.

reduction of fungal decay described previously (Section 3.1.2)

Many factors contribute to fruit texture. Holcroft and Kader (1999) described that the use of CO_2-enriched atmospheres during storage delayed strawberries softening. Other authors have reported that firmness was not only maintained but also enhanced by treatment with CO_2 (Harker et al., 2000). The results shown in this work suggest that firmness was highly maintained in fruit that were heat-treated in the presence of the film with lower permeability to CO_2 such as PD961EZ. This combination allowed adding the benefit of CO_2 retention to the well-known effect of heat treatment on delaying fruit softening.

3.2. pH and titratable acidity

The pH of the strawberry juice was not significantly modified under any of the conditions analyzed (data not shown). The results are in agreement with previous work with strawberry cv. Selva, in which the pH of fruit did not change after storage in CO_2-enriched atmospheres (Gil et al., 1997) or heating at 45 °C for 3 h (Vicente et al., 2002).

No difference in titratable acidity was found immediately after the heat treatment; however, after 2 days at 20 °C, control fruit in PVC film had more advanced values of titratable acidity than the corresponding heat-treated fruit ($P < 0.05$) (Table 1).

After 7 days at 0 °C, there were no significant modifications in fruit acidity levels relative to the initial values, but when the fruit were transferred to 20 °C the acidity in control fruit increased. After 14 days at 0 °C plus 2 days at 20 °C, the PVC control had higher acidity than the corresponding heat-treated fruit. The control fruit acidity showed a tendency to rise during the stay at 20 °C, as tissue disruption and fruit damage took place.

3.3. Sugar content

The total sugar content was slightly decreased just after heat treatment in PVC-packed, fruit, but there were no significant differences among treatments (Table 1). Different results were found by García et al. (1995) who observed that hot water treatments could improve the soluble solids content of strawberries. During the storage period, total sugar content decreased in all the conditions analyzed. After 2 days at 20 °C, strawberries in PD961EZ, which initially accumulated more CO_2 than the control, maintained more advanced levels of total sugars. After 7 or 14 days at 0 °C and further incubation at 20 °C, the total sugar content continued decreasing and no significant differences were found between control and treated fruit.

3.4. Total phenols

Immediately after the heat treatment and after 2 days at 20 °C, there were no significant differences among treatments (Table 1). However, when the fruit in PVC film were refrigerated for 7 or 14 days at 0 °C and then for 2 days at 20 °C, control fruit had higher total phenols than treated fruit ($P < 0.05$) (Table 1). No significant difference was found between control and treated fruit that were held PD961EZ film. Gil et al. (1997) reported that quercetin and kaempferol content increased in strawberries during storage. In addition, they found that ellagic acid was low in fruit stored in CO_2-enriched atmospheres. The increase in total phenols in control fruit during storage could be due to fruit damage and tissue disruption that takes place during storage. Phenolic compound synthesis in response to wounding has been reported (Saltveit, 2000). The increase in phenolic compounds was higher in control fruit in PVC film, which was the condition that showed more damage when the visual appearance was evaluated.

3.5. Assay II (fruit with 90% red color)

In this case, a perforated film (PY8) and a non-perforated film (PD961EZ) were used. The levels of CO_2 inside the trays in PD961EZ were similar to those described in the previous experiment, while the storage atmosphere corresponding to fruit in perforated film was air.

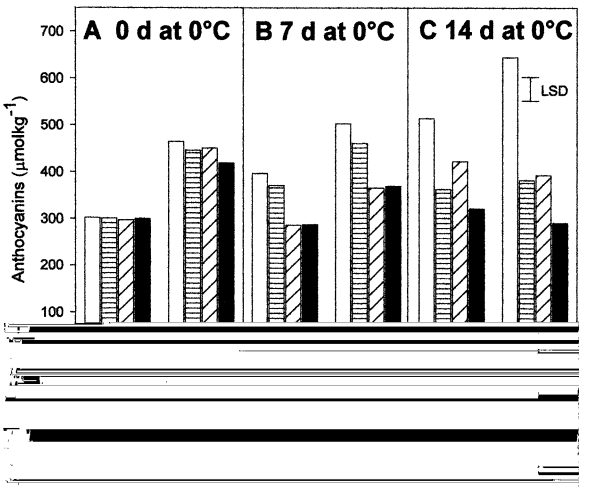

Fig. 5. Changes in anthocyanin content in control and heat-treated fruit harvested when 90% red, sealed in PY8 (perforated) or PD961EZ film, during storage at 0 °C and subsequent storage at 20 °C. Error bar indicate the LSD at $P = 0.05$.

In this experiment the same measurements than as Assay I were done. Most of the evaluated parameters presented a similar trend as in the case of 75% red fruit, and only the results concerning anthocyanins and fruit decay are shown and described. The results for anthocyanin content are shown in Fig. 5. After heat treatment, no differences were found in anthocyanin content in any condition. When the fruit were stored for 7 or 14 days at 0 °C and transferred to 20 °C for 2 days, heat-treated fruit had lower anthocyanin content than the respective controls ($P < 0.05$).

In the case of decay, both groups of heat-treated fruit had less decay than controls during the stay at 20 °C (Fig. 6A). In turn, heat-treated fruit in PD961EZ film had 7.5% of decayed fruit after 2 days at 20 °C while in PY8 film had 25% infected fruit. After 7 days at 0 °C and subsequent storage at 20 °C, a similar trend was found, with heat-treated fruit packed with PD961EZ film having lower decay percentage (Fig. 6B). After 14 days at 0 °C and 1 day at 20 °C similar results were observed, but thereafter the decay increased dramatically in all the conditions analyzed and no significant differences were observed (Fig. 6C).

4. Conclusions

These experiments demonstrate a benefit from a short-term heat treatment after fruit packaging.

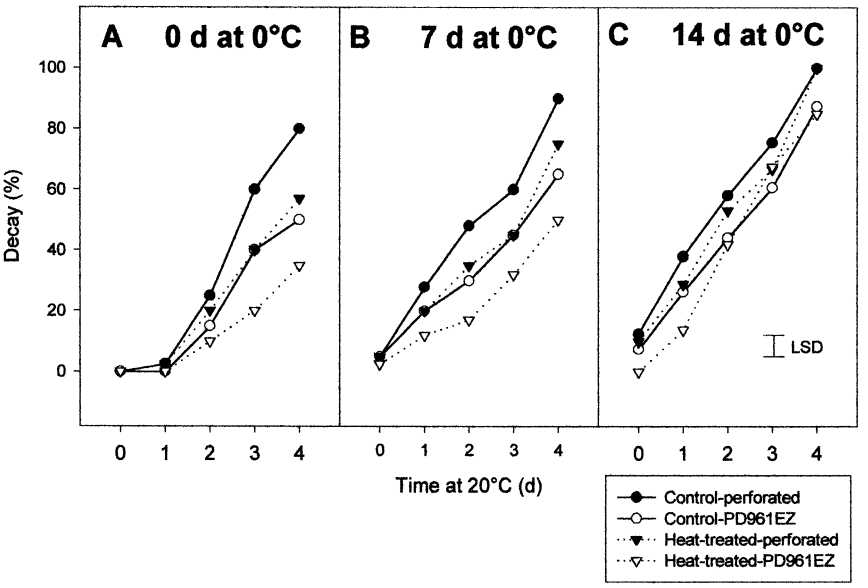

Fig. 6. Changes in decay in control and heat-treated fruit harvested when 90% red, sealed in PY8 (perforated) or PD961EZ film, during storage at 0 °C and subsequent storage at 20 °C. Error bar indicate the LSD at $P = 0.05$.

The main beneficial effects due to application of heat treatment in strawberries are decay reduction and delay in fruit softening and color development. These benefits are enhanced by performing the treatment on fruit covered with films that allow an initial retention of the CO_2 produced by the rise in fruit respiration during heating. Both effects of the combined treatment remain after storage at 0 °C. The benefit for 90% red fruit was lower than for 75% red fruit and decay development was only partly delayed. More research on the combination of CO_2 enriched atmospheres and heat treatment is needed to optimize the methodology.

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