

Polyamines, Ethylene, and Physicochemical Changes in Low-Temperature-Stored Peach (*Prunus persica* L. Cv. Maycrest)

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Fruit firmness, free putrescine and spermidine levels, total soluble solids, titratable acidity, and ethylene production were determined in two ripening grade Maycrest peaches stored at 1 and 5 °C and when fruits were placed at 20 °C during 48 h. Storage at 1 °C of peaches resulted in significantly longer postharvest life than did storage at 5 °C. Titratable acidity of fruit stored at 5 °C tended to decrease during storage and soluble solids concentration to increase. Peaches maintained at 1 °C had lower ethylene emission than those stored at 5 °C. Fruits in stage 2 that were placed at 20 °C had the highest ethylene levels and they became over-ripe. A close and inverse relationship has been observed between ethylene and firmness. Putrescine and spermidine concentrations evolved in a similar way during storage at 1 and 5 °C in stages 1 and 2 and decreased in the fruits kept for 48 h at 20 °C except for stage 2 peaches stored at 5 °C, for which the spermidine levels increased. These fruits showed wooliness and became inedible. The increase observed in spermidine levels could be a consequence of this kind of stress.

Keywords: Peach; *Prunus persica*; polyamines; ethylene; firmness; postharvest

INTRODUCTION

In peach fruit, the ripening process is typically divided into two stages: in the early phase, softening proceeds slowly, whereas in the second phase, the loss of firmness is rapid and associated with a dramatic increase of polygalacturonase activity (Downs et al., 1992; Orr and Brady, 1993). During peach fruit ripening, an autocatalytic ethylene biosynthetic pathway is activated and peaches show a climacteric peak of ethylene production, which seems to be related to the loss of firmness and other chemical modifications, such as a soluble solids concentration (SSC) increase and a decrease of the acidity (Miller et al., 1988; Amorós et al., 1989; Tonutti et al., 1996). This ripening process quickly increases after the autocatalytic ethylene production starts, and the fruits lose their commercial quality in a short period of time. To solve this problem, fruit is usually harvested in the early stage of ripening.

Low-temperature storage immediately after harvest is a common technique used to extend the storage life of fruit. However, many fruits and vegetables exposed to low but nonfreezing temperatures (below 12 °C) show a variety of symptoms that include uneven and abnormal ripening, increase in water loss, surface pitting, ethylene and CO₂ production, etc., that are known as chilling injury (CI) symptoms (Saltveit and Morris, 1990). In chilling-sensitive crops, CI becomes more severe during storage for long periods at low temperatures. There are also differences in chilling sensitivity in fruit at different maturity stages (Saltveit and Morris, 1990; Lin et al., 1993; Serrano et al., 1995a). In peach and other stone fruits, the limiting factor of their storage is the development of internal browning and lack of

juiciness (wooliness, dryness, mealiness) of the pulp, which are some of the CI symptoms of peach (Nanos and Mitchel, 1991; Luza et al., 1992).

Exposure to low temperatures induces biochemical and physiological changes in vegetable tissues, such as increases in polyamine levels. It has been proposed that polyamines may be involved in reducing CI, since fruit submitted to pretreatments which increase the endogenous levels of polyamines has more resistance to CI (Serrano et al., 1996). Little is known about the key role of polyamines in fruit ripening, and controversial results have been obtained. In avocado, apple, and pepper, free polyamine levels decreased during fruit ripening (Winer and Apelbaum, 1986; Biasi et al., 1988; Serrano et al., 1995b). In contrast, in orange and cherimoya these increased during the process (Hasdai et al., 1986; Escribano and Merodio, 1994). In tomato, putrescine decreases in the fast-ripening varieties and increases in long-keeping varieties, which may account for the slow ripening and low ethylene production of these fruits (Casas et al., 1990; Saftner and Baldi, 1990; Martínez-Madrid et al., 1996).

In this paper the possibilities of conservation of peach at two maturity stages (stage 1, unripe; and stage 2, ripe but preclimacteric), under two different temperatures (1 and 5 °C), are reported as are the physicochemical and physiological modifications during cold storage and after transfer to 20 °C.

MATERIALS AND METHODS

Plant Material. Peach (*Prunus persica* L. cv. Maycrest) fruit was harvested from commercial farms in Murcia (Spain) at two stages of ripeness: stage 1, unripe; and stage 2, ripe. Some of the characteristics of those ripening stages were as follows: firmness, 63 N for stage 1 and 37 N for stage 2; ethylene production, 0.95 and 3.76 nL g⁻¹ h⁻¹ for stages 1 and 2, respectively; SSC, 10.32 and 12.83, respectively; and titratable acidity (TA), 0.98 and 0.78 g 100 g⁻¹ for stages 1 and 2, respectively. At the laboratory, 50 fruits of each stage of maturity were kept at 1 °C and 50 fruits at 5 °C, in two

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temperature-controlled chambers, in permanent darkness and with a relative humidity of 90%. Every week 10 fruits of each ripening stage and storage temperature were sampled and placed at 20 °C. Five of these fruits were sampled for ethylene determination after 2 h, and the others were kept at 20 °C for 48 h.

Ethylene Production. The ethylene production rate was measured by placing each fruit in a 0.5-L glass jar hermetically sealed with a rubber stopper for 1 h. One milliliter of the holder atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a GC equipped with a flame ionization detector and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. The results were expressed in nanoliters of ethylene released per gram of tissue per hour ($\text{nL g}^{-1} \text{h}^{-1}$). After the determination of ethylene levels, two cylinder specimens were removed from each fruit to determine firmness. The peaches were then sliced to obtain a homogeneous sample of each one, and SSC and TA were determined. Afterward, the slices were individually frozen in liquid N_2 and stored at -20 °C until polyamines were analyzed.

Fruit Firmness. The assays for mechanical characteristics quantification were made on cylindrical specimens of 1 cm height and 1 cm diameter, using two samples obtained from opposite sides of each peach. The samples were compressed until full breakdown, using a Lloyd LR 5K universal assay machine (Lloyd Instruments). The displacement rate was fixed at 20 mm min^{-1} . The maximum strength at the time of breakthrough was determined. Results were expressed in newtons.

TA and Total SSC Determination. TA was determined by potentiometric titration with 0.1 N NaOH up to pH 8.1 using 1 mL of diluted juice in 25 mL of distilled H_2O . The results were expressed as grams of malic acid per 100 g of fresh weight. Two measurements were made from each fruit. Total SSC was determined by a P20 RL2 refractometer at 20 °C. Three determinations were made from each fruit.

Polyamine Analysis. Free polyamines were analyzed according to the method of Flores and Galston (1982). Two extractions of polyamines were made from each peach. One gram of fresh tissue was extracted with 10 mL of 5% cold perchloric acid. 1,6-Hexanediamine (100 nmol g^{-1}) was added as internal standard. The homogenate was then centrifuged for 30 min at 20000g. Free polyamines left in the supernatant were benzoylated as previously described (Serrano et al., 1995b). Derivatives were analyzed by HPLC. The elution system consisted of MeOH/ H_2O (64:36) solvent, running isocratically with a flow rate of 0.8 mL min^{-1} . The benzoyl polyamines were eluted through a reversed-phase column (LiChroCart 250-4, 5 μm) and detected by absorbance at 254 nm. A relative calibration procedure was used to determine the polyamines in samples, using 1,6-hexanediamine as the internal standard and standard curves for putrescine, spermidine, and spermine.

Statistical Design. Experimental data are the mean \pm SE of the determinations for each sample. A variance analysis using the Student *t*-test was performed to determine if the comparison between ripening stages and storage temperatures shows significant differences ($p < 0.05$).

RESULTS

Firmness, SSC, and TA in Stored Peaches. Initial values of firmness were 63 and 37 N in stages 1 and 2, respectively. For both, firmness levels remained unchanged during the storage at 1 °C (Figure 1a,c), while in the fruits stored at 5 °C, firmness levels quickly decreased during the first 2 weeks for stage 1 and during the first week for stage 2 (Figure 1b,d). When fruits stored at 1 and 5 °C were placed at 20 °C, firmness notably decreased. Fruits in stage 1 stored at 1 °C and placed at 20 °C reached firmness levels near the initial levels of those of stage 2. However, for peaches in stage 2 stored at 1 °C, the firmness levels reached after transfer to 20 °C were very low, near 5 N (Figure 1a,c).

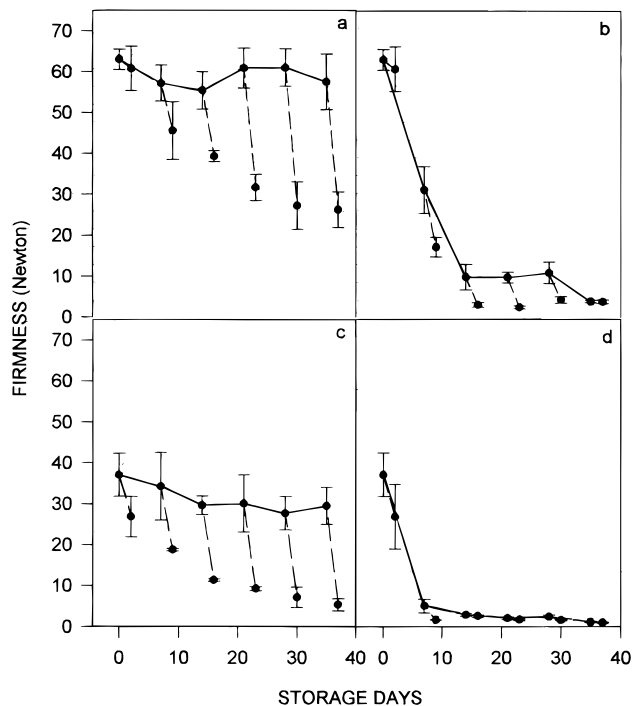


Figure 1. Firmness changes (newtons) in cold-stored peaches: (a) stage 1 fruit stored at 1 °C; (b) stage 1 fruit stored at 5 °C; (c) stage 2 fruit stored at 1 °C; (d) stage 2 fruit stored at 5 °C; (solid line) firmness (newtons) of fruits placed at 20 °C for 48 h; (broken line) mean \pm SE of 10 determinations.

The values of SSC in the fruits just harvested were 10.32 ± 0.72 and 12.83 ± 0.53 for stages 1 and 2, respectively, and remained constant during the 5 weeks of the experiment at 1 °C. They significantly increased when placed for 2 days at 20 °C, reaching levels of 12.75 ± 0.64 and 13.94 ± 0.34 in stages 1 and 2, respectively, after 5 weeks of storage at 1 °C and 2 days at 20 °C. Peaches stored at 5 °C showed a significant increase of SSC, reaching 12.81 ± 0.24 for stage 1 and 14.20 ± 0.32 for stage 2 fruits after 5 weeks of storage. When these fruits were placed at 20 °C for 2 days, a slight decrease in SSC took place (although it was only significantly different in some samples), which might be attributed to sugar consumption during cellular respiration in these over-ripe fruits.

TA levels were 0.98 ± 0.03 and $0.78 \pm 0.04 \text{ g } 100 \text{ g}^{-1}$ for stages 1 and 2, respectively. These levels were stable during storage at 1 °C but decreased significantly for storage at 5 °C. After 5 weeks of storage at 5 °C, values of 0.56 ± 0.04 and $0.39 \pm 0.02 \text{ g } 100 \text{ g}^{-1}$ appeared for fruits in stages 1 and 2, respectively. When fruit stored at 1 °C was taken out for 2 days at 20 °C, the TA levels significantly decreased for both fruit stages, and values of 0.75 ± 0.07 and 0.51 ± 0.04 were obtained in stages 1 and 2, respectively, after 5 weeks of storage at 1 °C and 2 days at 20 °C. In fruits stored at 5 °C TA levels decreased slightly when they were transferred at 20 °C, but these modifications were not significantly different at the $p < 0.05$ level.

Ethylene Production in Stored Peaches. Ethylene production was very low during storage at 1 °C, in stages 1 and 2 (Figure 2a,c), and it rose in both during storage at 5 °C (Figure 2b,d). This increase was more relevant from the second week of storage in stage 1 and from the first week in stage 2 (Figure 2b,d). A considerable increase in the ethylene production was observed when peach fruit (stage 1) stored at 1 °C for 3, 4, and 5 weeks was transferred to 20 °C, reaching levels of 12.4,

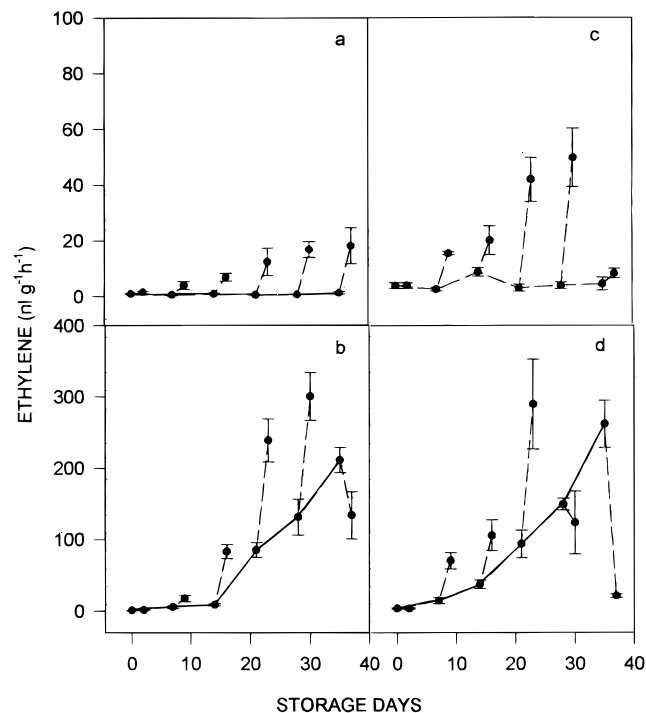


Figure 2. Ethylene production rate ($\text{nL g}^{-1} \text{h}^{-1}$) in cold-stored peaches: (a) stage 1 fruit stored at $1\text{ }^{\circ}\text{C}$; (b) stage 1 fruit stored at $5\text{ }^{\circ}\text{C}$; (c) stage 2 fruit stored at $1\text{ }^{\circ}\text{C}$; (d) stage 2 fruit stored at $5\text{ }^{\circ}\text{C}$; (solid line) ethylene production rate ($\text{nL g}^{-1} \text{h}^{-1}$) of fruits placed at $20\text{ }^{\circ}\text{C}$ for 48 h; (broken line) mean \pm SE of five determinations.

16.8, and $18.2\text{ nL g}^{-1} \text{h}^{-1}$, respectively (Figure 2a). In this figure the beginning of the climacteric stage is also shown.

When the stage 2 fruits stored at $1\text{ }^{\circ}\text{C}$ were placed at $20\text{ }^{\circ}\text{C}$, the increment of ethylene production was higher, reaching the climacteric peak after 4 weeks at $1\text{ }^{\circ}\text{C}$ and 2 days at $20\text{ }^{\circ}\text{C}$, with an ethylene production of $49.7\text{ nL g}^{-1} \text{h}^{-1}$ (Figure 2c). After storage at $5\text{ }^{\circ}\text{C}$ and 2 days at $20\text{ }^{\circ}\text{C}$, ethylene production increased (Figure 2b,d). However, this increase cannot be attributed to a climacteric rise but rather to an over-ripe ethylene production due to a postclimacteric stage. These fruits were over-ripe and consequently not suitable for consumption, since firmness was very low (Figure 1b,d).

Polyamine Levels of Stored Peach. Free polyamine levels were analyzed in peach fruit, but only putrescine and spermidine were quantified, since spermine levels were not detected. No differences were found in putrescine levels between fruits in stage 1 stored at 1 and $5\text{ }^{\circ}\text{C}$. The putrescine levels increased during storage, reaching maximum values at the third week (Figure 3a,b), and decreased from this time. For stage 2, initial levels of putrescine were 52.12 nmol g^{-1} of fresh weight, almost double those in stage 1 when harvested. They decreased during the first weeks of storage at 1 and $5\text{ }^{\circ}\text{C}$ but then increased until they reached the maximum value at the third week (Figure 3c,d). When all of the fruit was taken out after 2 days at $20\text{ }^{\circ}\text{C}$, the putrescine levels decreased, reaching levels as low as 2.27 nmol g^{-1} of fresh weight (Figure 3).

In stage 1, spermidine levels were $364.16\text{ nmol g}^{-1}$ of fresh weight at the beginning of the experiment and fell during the next 2 weeks of storage at 1 and $5\text{ }^{\circ}\text{C}$, reaching then values of 57.19 and 48.16 nmol g^{-1} of fresh weight, respectively. From this time on, an increase of spermidine was found in peaches stored at both temperatures (Figure 4a,b). When fruits were

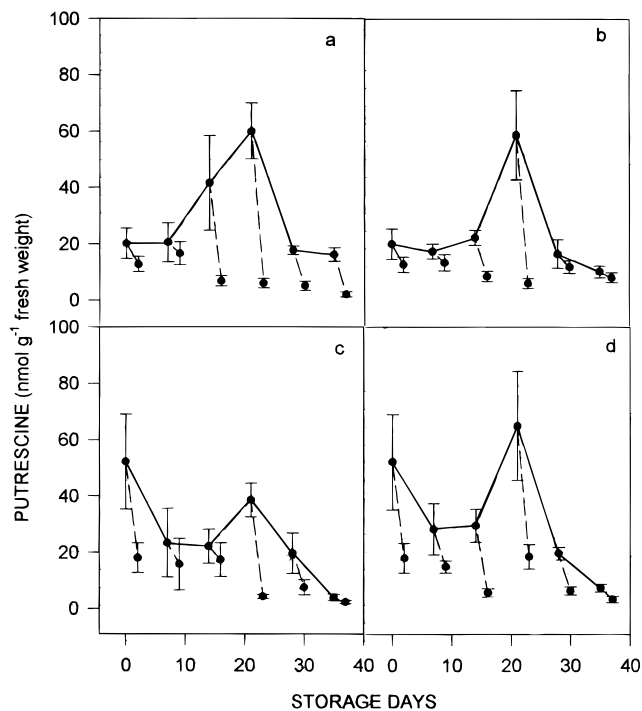


Figure 3. Putrescine levels (nmol g^{-1} of fresh weight) in cold-stored peaches: (a) stage 1 fruit stored at $1\text{ }^{\circ}\text{C}$; (b) stage 1 fruit stored at $5\text{ }^{\circ}\text{C}$; (c) stage 2 fruit stored at $1\text{ }^{\circ}\text{C}$; (d) stage 2 fruit stored at $5\text{ }^{\circ}\text{C}$; (solid line) putrescine levels (nmol g^{-1} of fresh weight) of fruits placed at $20\text{ }^{\circ}\text{C}$ during 48 h; (broken line) mean \pm SE of 10 determinations.

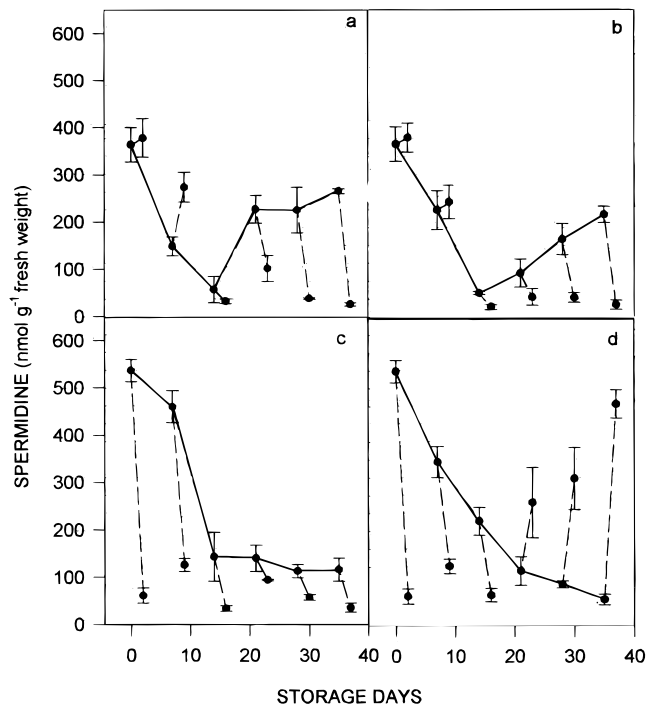


Figure 4. Spermidine levels (nmol g^{-1} of fresh weight) in cold-stored peaches: (a) stage 1 fruit stored at $1\text{ }^{\circ}\text{C}$; (b) stage 1 fruit stored at $5\text{ }^{\circ}\text{C}$; (c) stage 2 fruit stored at $1\text{ }^{\circ}\text{C}$; (d) stage 2 fruit stored at $5\text{ }^{\circ}\text{C}$; (solid line) spermidine levels (nmol g^{-1} of fresh weight) of fruits placed at $20\text{ }^{\circ}\text{C}$ during 48 h; (broken line) mean \pm SE of 10 determinations.

placed at $20\text{ }^{\circ}\text{C}$ for 48 h, a slight increase was found during the first 2 weeks and then a decrease from 3 to 5 weeks of storage.

In fruits of the stage 2, initial values of spermidine were higher ($536.78\text{ nmol g}^{-1}$ of fresh weight) than the levels found in stage 1. Nevertheless, a strong decrease

was observed during the first 2 weeks of storage at both temperatures. From the third week on, the decrease in the levels of spermidine was less pronounced (Figure 4c,d). This behavior is different from that found during storage of peaches in stage 1. In both ripening stages, an initial diminution of spermidine levels was observed that leveled off slightly from the third week in stage 2 and increased in stage 1.

DISCUSSION

Results of firmness evolution during storage showed that peach in stage 1 can be stored for 5 weeks at 1 °C. These fruits, after being placed at 20 °C for 2 days, reached firmness values similar to those of fruit in stage 2 peach just harvested. In addition, fruits harvested in stage 2 can also be stored at 1 °C for 5 weeks without modification in their firmness. However, when these fruits were placed at 20 °C for 2 days, firmness decreased to very low levels and the fruit showed both texture and flavor characteristics unacceptable after storage for more than 2 weeks. The same results have been reported in other cultivars of peach and in nectarines kept at 0 and 5 °C for more than 2 weeks (Bramlage, 1982). The 5 °C storage temperature was not low enough to stop the metabolic processes involved in fruit ripening. At this temperature, only stage 1 fruits can be stored, and then only for 1 week, since after that time firmness is too low and the fruits become inedible. These results show that when peach firmness is <12–15 N, its texture is too soft and the fruit does not have acceptable characteristics for consumption.

CI symptoms were not detected in peaches stored at 1 °C or in stage 1 fruits stored at 5 °C, but stage 2 peaches kept for more than 2 weeks at 5 °C developed wooliness when they were transferred to 20 °C. Wooliness is characterized by a dry, mealy texture and loss of flavor and brightness of color (Bramlage, 1982), and it seems primarily to be associated with the cell wall structure and inversely correlated with Ca contents in the fruits (Von Mollendorff et al., 1992). These results are in agreement with those of Nanos and Mitchell (1991), who found that the 5 °C temperature was the worst for stone fruit storage (peaches and nectarines) since it maximized internal breakdown, browning, and mealiness. Results also show that picking maturity determines wooliness development, since only stage 2 fruits showed this CI symptom. This process could be different depending of the variety studied, since in other fruits no differences were found in CI symptoms over a range of fruit maturity stages and they are also results showing that the more immature the peach was when it was picked, the higher the incidence of wooliness was (Von Mollendorff et al., 1992).

SSC and TA levels, like firmness results, indicate that during the 1 °C storage the ripening process was totally inhibited, while at 5 °C the process followed. For both stages 1 and 2 peaches, firmness and TA decreased and SSC increased. However, when peaches were taken out at 20 °C, these modifications appeared very quickly. Only stage 1 peaches stored at 1 °C for 5 weeks and stage 2 peaches stored during 1 week showed levels of these parameters acceptable for consumption.

A close and inverse relationship between firmness and ethylene production levels in peach fruit stored at 1 °C could be observed. From these results it can be inferred that firmness had to decrease to near 30 N for ethylene production to start, and the climacteric peak was reached when firmness levels were near 10 N (Figure

1a,c; Figure 2a,c). This increase in ethylene production has been associated with the physicochemical changes that occur during the peach ripening (Amorós et al., 1989). In the storage experiment at 5 °C, firmness levels reached when the fruits were taken out at 20 °C were very low, except for stage 1 after 1 week of storage. The high values of ethylene emission found could be attributed to an over-ripening ethylene rather than to a climacteric ethylene (Figure 1b,d; Figure 2b,d). In other peach varieties, it has been found that the climacteric peak of ethylene production occurred when the fruit had already softened to about 10–20 N (Tonutti et al., 1996). These results clearly indicate that, in the tested cultivars, the climacteric is a late event occurring after the fruit has consistently softened. Since the eating quality of commercial peach varieties is higher at firmness values of 30–40 N, peach should be listed among those fruit species in which the ethylene climacteric coincides with (avocado, banana) or follows (kiwi) the eating ripeness stage (Amorós et al., 1989; Tonutti et al., 1996). Whether some of the changes occurring during the early stage of ripening are due to a small stimulation of ethylene biosynthesis and/or an enhanced tissue sensibility to the hormone remains to be elucidated.

There was a clearly different behavior in spermidine evolution between the two storage temperatures tested for peach in stage 2 when they were transferred to 20 °C. The spermidine levels decreased in peaches that had been stored at 1 and 5 °C for 1–2 weeks but increased in those stored at 5 °C for 3–5 weeks. We had noticed that these fruits showed an increase in wooliness and the fruits were much softer. Mesocarp wooliness is one aspect of CI that appears after fruits are held at room temperature for a few days following cold storage at 5 °C, in accordance with the results of Luza et al. (1992) obtained with other peach cultivars. Our results have shown that wooliness is related to the fruit ripening grade, since it has only been detected in peaches in a highly advanced ripening stage. This has also been reported on apples and could be associated with low adhesion between neighboring mesocarp and parenchyma cells (Harker and Hallett, 1992).

The results showed that no differences were found in the evolution of putrescine and spermidine during peach storage at 1 and 5 °C which could be attributed to the different storage temperatures. This is consistent with the absence of chilling symptoms observed in the fruits and agrees with the results of other researchers (Serrano et al., 1996, and references cited therein) on the fact that only the plant organs susceptible to CI had increased putrescine and/or spermidine levels during low-temperature storage. Only in peach fruit that showed wooliness has an accumulation of spermidine been found, which could be related to this kind of stress.

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