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Heat and anaerobic treatments affected physiological and biochemical parameters in tomato fruits

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Abstract

The aim of the present work was to evaluate the effect of thermal and anaerobic treatments on physiological and biochemical parameters in a variety of tomatoes (*Lycopersicon esculentum* Mill. cv. Colt 45). Treatments applied to mature green tomatoes were: (A) heat treatments by water immersion at 42 °C for 30 min (HS30′) or for 60 min (HS60′); or by air at 38 °C for 72 h (HS72h); and (B) anaerobic treatments carried out at 20 °C under humidified nitrogen atmosphere for 3 days (ANA3d) or 6 days (ANA6d). After treatments, fruits were stored at 2 or 14 °C. Parameters evaluated were: colour, total acidity, major organic acids, firmness, and ethanol and acetaldehyde concentration. Anaerobic and long-term heat shock treatments inhibited colour development irrespective of storage temperature. Air heat treatment reduced tritratable acidity by increasing malic acid metabolism. Anaerobic treatments induced ethanol accumulation, which could be reversed during storage for the short treatment (3 days), but not for the longer treatment (6 days). Acetaldehyde concentration was increased by anaerobic treatments, but also by immersion in hot water for 60 min, which would produce a ''low-aerobic'' environment.

Keywords: Chilling injury; Heat shock; Stress; Anaerobiosis; Organic acids

1. Introduction

Chilling injury is a physiological disorder caused by the exposure of fruits to low temperatures above the freezing point, generally resulting in quality reduction (Parkin, Marangoni, Jackman, Yada, & Stanley, 1989). Many researchers have reported the use of high temperatures (thermal shock) to maintain quality and extend fruit shelf life (Murray, 1992; Sabehat, Weiss, & Lurie, 1996; Lurie, 1998). Tomato fruits exposed to high-temperature stress did not develop the characteristic symptoms of chilling injury after a storage of 3 weeks at 2 °C (Lurie & Klein, 1991; Lurie et al., 1993). Heat treatments were also successful in other commodities like strawberries (Civello, Martínez, Cháves, & Añón, 1997), avocado (Florissen et al., 1996) and apples (Lurie & Klein, 1990).

Several authors have established a relationship between the heat-shock response and the protection exerted against different types of stress. According to Schoffl, Prandl, and Reindl, (1998) the heat stress induces a cellular response that is able to protect both the cell itself and the whole organism from severe damage. Bierkens (2000), and Neumann, Lichtenberger, Gunther, Tschiersch, and Nover (1994) described that a single stress (dehydration, heavy metals, oxidative stress, etc.) induced a response able to exert a cross protection against any other stress. We speculate that the application of anaerobic stress, used at sub-lethal levels, could

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also have the potential of preventing physiological disorders in fruits and vegetables. The application of N_2 before storage has been shown to delay fruit ripening, reduce chilling injury, and extend avocado storage life (Pesis, Marinansky, Zauberman, & Fuchs, 1993). However, if these anaerobic treatments are not properly used, it could be detrimental for fruits. The injuries produced by low oxygen concentrations are highly dependent on the extent of the anaerobic conditions and, when fruits are earlier restored to aerobic conditions, there is a possibility of preventing permanent damage (Kays, 1997).

Considering that much of the knowledge on stress treatments is empirical, and there is a lack of publications comparing in the same research different types of stresses, the aim of the present work was to evaluate the effect of thermal and anaerobic stresses on physiological and biochemical parameters in a commercial variety of tomatoes (*Lycopersicon esculentum* Mill. cv. Colt 45).

2. Materials and methods

2.1. Plant material

Nine hundred and sixty mature-green tomatoes (USDA, 1976) of uniform size were picked directly from the greenhouse (Lurie & Klein, 1991, 1992). Fruits were first surface-sterilized for 3 min with a chlorine solution (150 mg/kg Cl_2 as sodium hypoclorite), then thoroughly rinsed with tap water for another 3 min, and finally left on filter paper to drain.

2.2. Stress treatments

The tomatoes were divided into six lots. Each lot was submitted to one of the following treatments:

I: No treatment, used as control = C.
II: Short heat shock treatment (immersion for 30 min in a water bath at 42 °C) = HS30′.
III: Short heat shock treatment (immersion for 60 min in a water bath at 42 °C) = HS60′.
IV: Long heat shock treatment (incubation in a traditional chamber at 38 °C and 95 percent relative humidity for 72 h) = HS72h.
V: Anaerobic treatment (incubation in a 20-l plastic chamber at 20 °C, with first a rapid atmosphere exchange by ventilation with humidified nitrogen at a flow rate of 100 ml/min for 2 h, and then a continuous influx of humidified nitrogen at 50 ml/min-flow rate for 3 d) = ANA3d.
VI: Anaerobic treatment (incubation in a 20 l plastic chamber at 20 °C, with first a rapid atmosphere exchange by ventilation with humidified nitrogen at a flow rate of 100 ml/min for 2 h, and then a continuous influx of humidified nitrogen at 50 cm^3/min-flow rate for 6 d) = ANA6d.

The experimental design included six treatments (C, HS30′, HS60′, HS72h, Ana3d, Ana6d); two storage temperatures per treatment (2 °C, 14 °C); one sampling immediately after treatment (S1), and two other sampling periods, 13 and 20 d after treatment and storage at each temperature (S2 and S3); two evaluation conditions for each sampling, immediately after sampling ($S1_0$, $S2_0$, $S3_0$) and, in order to induce ripening, fruits were also stored for additional 4 d in a chamber under normal atmosphere regulated at 20 °C ($S1_4$, $S2_4$, $S3_4$).

2.3. Colour measurements

Tomato colour measurement was performed with a Minolta Chroma Meter (CR 100 with 8 mm-aperture) equipped with data processor (DP-100, Minolta, Ramsey, NJ). Three readings of each value were carried out in different areas of each individual fruit. Measurements for individual fruit were made approximately 1 cm from the blossom scar. Hue value (h*), attribute for colour classification, was used as a ripening parameter. It starts at the (+) *a*-axis, and is expressed in degrees; 0° represents red magenta, 90° yellow, 180° green, and 270° blue. Mean values were obtained by analysis of four replications of four fruits each.

2.4. Total acidity (TA) determination

TA was determined in 10 cm^3 of tomato juice + 100 cm^3 of distilled water by titration with 0.1 mol/l sodium hydroxide to pH 8.3, and expressed as g citric acid/ 100 g tomato. Treatment mean values were obtained from four lots of four fruits each.

2.5. Organic acids analysis

Major organic acids in tomato juice were analysed by paper chromatography. Whatman no. 1 chromatographic paper was used as support. Solvent was composed of 900 cm^3/l *n*-butanol, 100 cm^3/l concentrated formic acid and 1.5 g/l of bromocresol green. Tartaric acid, citric acid and malic acid were used as standards.

2.6. Fruit firmness measurement

Firmness was measured at the equator on opposite sides of each individual fruit with a Shore A digitized durometer (DUROFEL 10 licensee by CTIFL-France). This is a nondestructive method used to measure the superficial firmness of soft fruits by applying an

external pressure without penetration. Measurement was expressed using a scale range of 1–100, with higher values for firmer fruits. Mean values were obtained by analysis of four replications of four fruits each. Two values were obtained per fruit.

2.7. Ethanol and acetaldehyde (AA) measurements

Concentrations of acetaldehyde and ethanol were determined by homogenizing 20 g of fruit with 100 cm^3 of a cold solution of hydrochloric acid (0.1 mol/l), incubating 10 cm^3 of homogenates in 20 cm^3 tubes sealed with teflon caps during 30 min at 70 °C, and injecting 2 cm^3 of the headspace in a HP 5890 series II gas chromatograph with a flame ionization detector and a Porapak Q column. Mean values were obtained by analysing four pools of four fruits each.

2.8. Statistical analysis

A completely randomized design was used. Each evaluation condition involved four replications of four fruits each. Significant differences were analysed by analysis of variance using the General Linear Model procedure of SAS software. When significant main effects were observed without interactions, mean separation was accomplished by the use of Duncan's Multilple Range Test and Least Significant Difference Procedure. The comparison was usually made between each treatment and the control (C). However, when required, comparisons among treatments were considered.

3. Results and discussion

3.1. Colour changes

Immediately post treatment and after storage at 2 °C (13 and 20 days) no significant differences were detected in h* values comparing each individual treatment against C (data not shown). At 14 °C and after 13 days of storage the colour of fruits submitted to long heat shock and anoxic treatments (HS72h, ANA3d, ANA6d) had a higher h value compared to C, an indication of a fruit ripening delay. After 20 days at 14 °C, ANA6d was the only treatment that showed significant differences compared to C (higher h values than C). C and the rest of the treatments had values from 45° to 50°, values that represent an orange-reddish colour (USDA colour stage 4). HS72h was not evaluated due to the decay of the complete lot.

Fig. 1 shows hue values for the different treatments measured at the three sampling periods: immediately after treatments ($S1_4$), after 13 days ($S2_4$) and after 20 days ($S3_4$) of storage at the indicated temperatures plus 4 days at 20 °C. For $S1_4$ sampling period, no differences were found in h values. Data obtained from fruits stored for 13 and 20 days at 2 °C ($S2_4$) showed that ANA3d and HS72h had higher h values (yellow-greenish color) than C. ANA6d treatment could not be evaluated due to the decay of the complete lot.

At 14 °C, ANA6d hue values were significantly higher than C for both storage periods ($S2_4$, $S3_4$). ANA3d h* value was higher than C after 13 days of storage ($S2_4$), however after 20 days of storage the fruit was able to attain the same level of colour development than C. The rest of the treatments presented hue values similar to C presenting values near 45° (orange-reddish colour–-USDA colour stage 5).

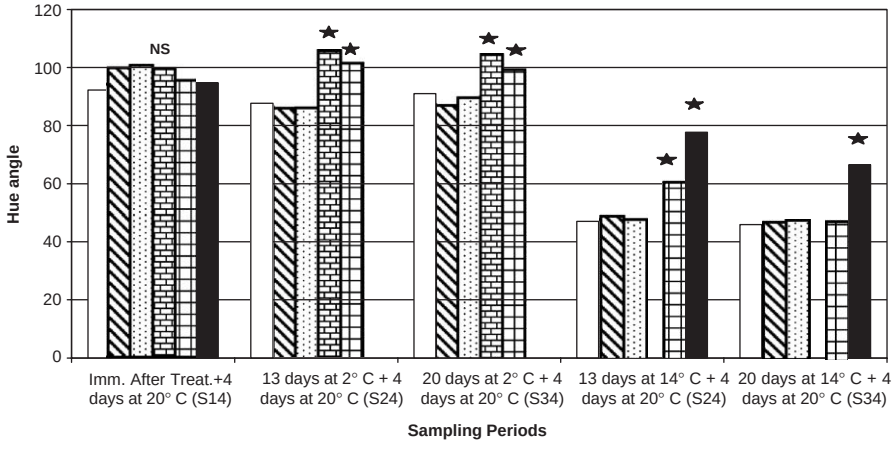

Fig. 1. Hue values of tomatoes untreated (Control), heated for 30 min (HS30′), 60 min (HS60′), or 72 h (HS72h), or subjected to anaerobiosis for 3 days (ANA3d) or 6 days (ANA6d). Fruits were analysed at the different sampling periods + 4 days of storage at 20 °C. NS: no significant differences ($P<0.05$) were found. Treatments significantly different ($P<0.05$) from control were marked with a star. Control □ HS30′ ▨, HS60′ ⊡, HS72h ⊞, ANA3d ▢, ANA6d ■.

Colour results make evident that ANA3d and HS72h produced ripening delay when fruits were stored at chilling temperature, while an extended anoxic treatment (ANA6d) was deleterious for the fruit. Holding the fruit at a higher temperature (14 °C) showed that ANA6d did not decay (probably due to a nonstressing temperature of storage) and a ripening delay could be detected.

The detected effect of anaerobic treatment on ripening delay is probably caused by fermentative metabolites like ethanol and acetaldehyde produced under this condition, though the ripening process can be restarted when they dissipate (Ratanachinakorn, Klieber, & Simons, 1999). An increased acetaldehyde concentration was reported to inhibit ethylene production and consequently delay chlorophyll degradation and lycopene synthesis (Saltveit & Mencarelli, 1988). Lurie and Klein (1991) concluded that, even though heat stress could produce inhibition of ripening, this inhibition was only maintained at low temperatures, thus when the fruit is removed and transferred to 20 °C ripening process continues. Our results showed that heat treated fruits transferred to 20 °C for 4 days have signs of ripening retardation, hence it is probable that submitting the fruit to longer periods than 4 days at 20 °C, a colour development similar to control would be achieved.

3.2. Firmness

Fruits analysed immediately after treatment or stored at 2 °C showed that a 72 h heat treatment and anoxia

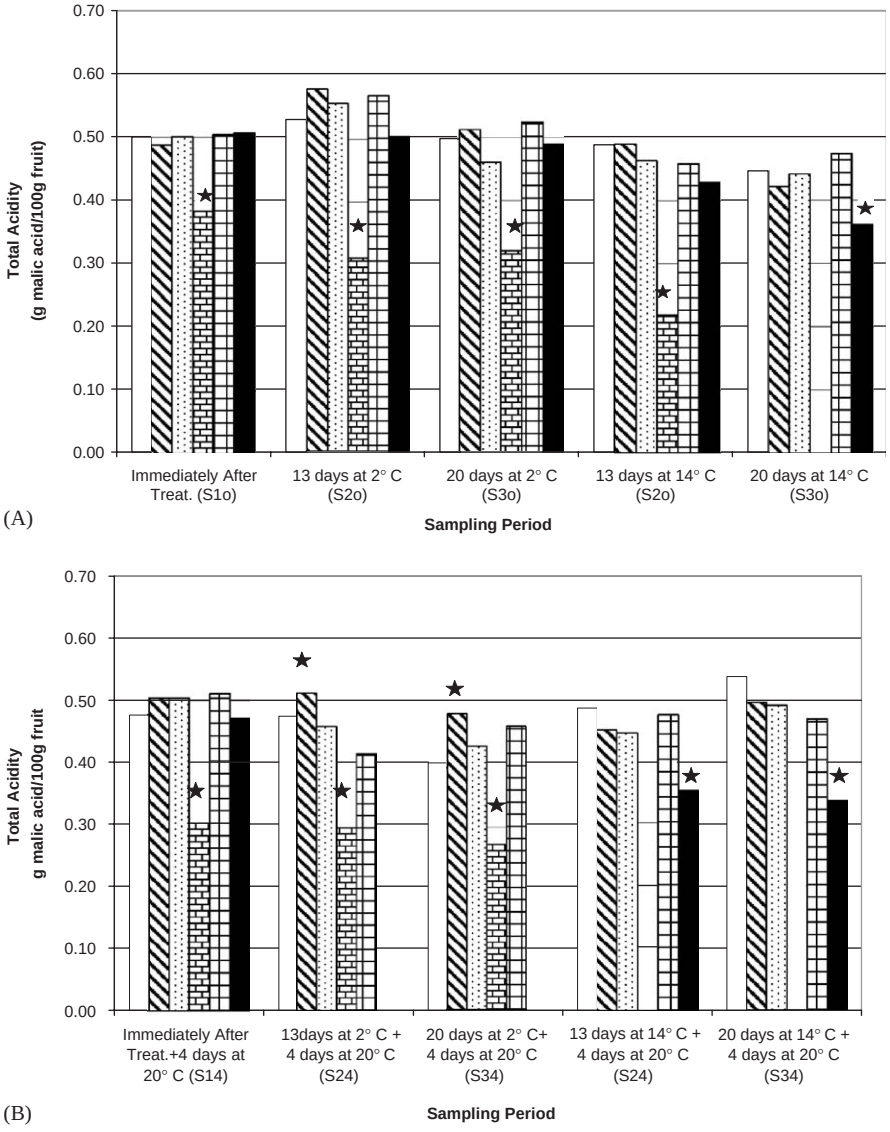

Fig. 2. Total acidity (g malic acid/kg fruit) of tomatoes untreated (Control), heated for 30 min (HS30′), 60 min (HS60′), or 72 h (HS72h), or subjected to anaerobiosis for 3 days (ANA3d) or 6 days (ANA6d). Fruits were analysed immediately after treatments or storage (A), or after 4 days at 20 °C (B). NS: no significant differences ($P<0.05$) were found. Treatments significantly different ($P<0.05$) from control were marked with a star. Control □ HS30′ ▨, HS60′ ⊡, HS72h ⊟, ANA3d ▢, ANA6d ■.

produced less firm fruits than the control, while short heat treatments produced a delay in fruit softening. No significant differences were found among treatments at 2 °C for any storage times. At 14 °C, no differences were found between C and the short-time heat treatments (HS30′ and HS60′) but higher firmness values were found in ANA 3d and ANA6d treatments after 13 days of storage, and in ANA6d after 20 days (data not shown).

Heat treatments were reported to delay fruit softening in several species like apples (Lurie & Klein, 1990), strawberries (Civello et al., 1997), and papaya (Paull & Chen, 1990). In tomatoes, Manzano–Mendez, Hicks, and Masters (1984) found that, although tomato softening is highly dependent on cultivar, when they are held at 35 °C and then moved to 20 °C, most of the cultivars tested became significantly softer than fruits maintained during the complete period at 20 °C. This is coincident with our finding for long heat treatments (HS72h), but not for short treatments (HS30′ and HS60′) (data not shown).

3.3. Total acidity

Fig. 2A, shows a significant decrease of TA values in fruits from HS72h treatment at the different sampling periods (S1–S3), and under both storage conditions (2 and 14 °C). This difference is pronounced and consistent. After holding the sampled fruit for another 4 days at 20 °C (Fig. 2B), the same result was found for fruit recently treated or stored at 2 °C. At 14 °C, ANA6d treatment was significantly lower than C at both evaluation conditions, with and without holding the fruit at ripening temperature.

To investigate which acid is involved in the acidity decrease, we analysed by chromatography the major organic acids found in tomatoes in control (C), and heat-treated samples (HS72h). The chromatogram (Fig. 3) made evident the reduced intensity of malic acid in HS72h samples. Consequently, it can be inferred that the decrease in TA found is mainly due to the increased metabolism of the malic acid. Citric acid concentration is rather unaffected by the heat treatment.

Under normal conditions, the predominant acid of ripened tomato fruits is citric acid, with malic acid being the next most abundant. Changes in TA have been attributed either to changes in citric acid alone, or to changes in both, citric and malic acids (Hobson & Davies, 1971). Lurie and Klein (1990) found that fruit respiration rate was greatly increased by heat treatment, and they postulated that the observed decrease in tomato acidity might be connected to the acceleration of the metabolism induced by the treatment. Malic acid is considered the first acid to be consumed in respiration (Tucker, 1993), which is in agreement with our results. Thus, it can be concluded that air heat treatment

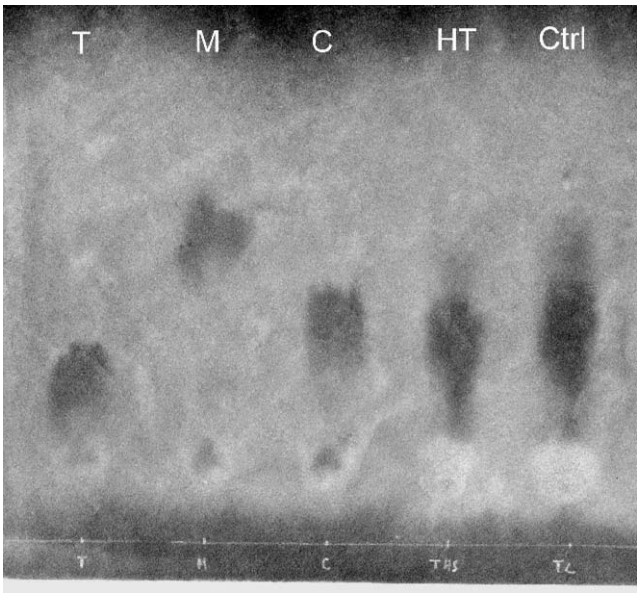

Fig. 3. Chromatographic analysis of main organic acid from tomato T: tartaric acid, M: malic acid, C: citric acid, HT: heat-treated fruit (72 h at 39 °C), Ctrl: control (untreated) fruit.

induces an increase of the basal metabolism accelerating particularly the consumption rate of this organic acid. To our knowledge, this is the first research that specifically determined the main acid to be metabolized in a heat treatment. The application of an air heat treatment will probably affect sensory characteristics by rendering a less acid fruit, which could also be a problem if further processing is conducted.

3.4. Acetaldehyde and ethanol content

Fig. 4 shows the ethanol concentration obtained in the six different treatments. Immediately after treatment (Fig. 4A), the anaerobic treatments (ANA3d and ANA6d) produced a significant increment (approximately 100 times) of ethanol concentration compared to C. A slight but nonsignificant increment was detected in short heat shock treatments achieved by water-immersion (HS30′ and HS60′). This effect could be due to the enhanced metabolism induced by water temperature (42 °C) and by the local anaerobic condition produced by the immersion. However, when the fruit was kept for 4 days at 20 °C (Fig. 4B), the ethanol concentration of ANA3d sample analysed immediately after treatment diminished, probably because of the removal of fruits from anaerobic conditions. Under more extended anaerobic conditions (ANA6d), the irreversibility of the metabolic reactions and/or the beginning of fruit decay would be probably responsible for the highest ethanol production.

Fig. 4A also shows that fruits stored at 2 °C for the two sampling periods ($S2_0$ and $S3_0$) had similar ethanol content patterns. At this temperature, only anaerobic

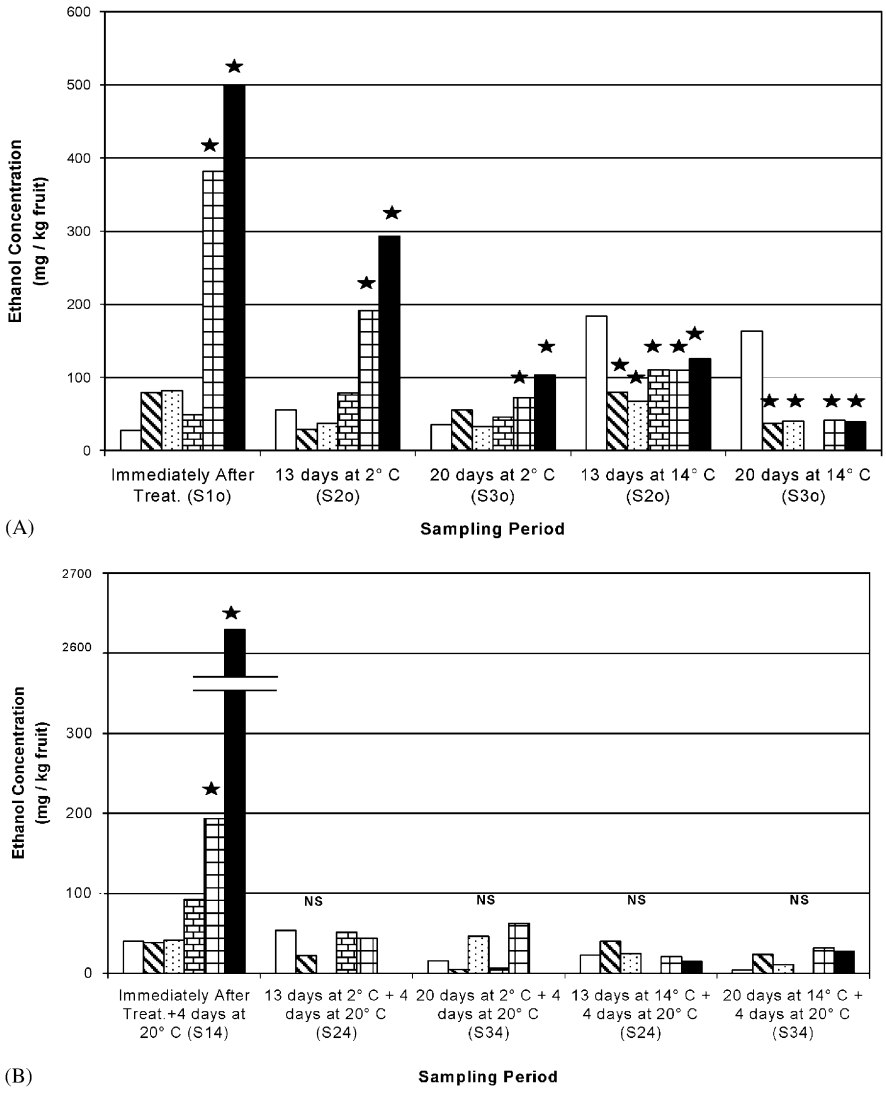

Fig. 4. Ethanol concentration (mg/kg of fruit) in tomatoes untreated (Control), heated for 30 min (HS30′), 60 min (HS60′), or 72 h (HS72h), or subjected to anaerobiosis for 3 days (ANA3d) or 6 days (ANA6d). Fruits were analysed immediately after treatments or storage (A), or after 4 days at 20 °C (B). NS: no significant differences ($P<0.05$) were found. Treatments significantly different ($P<0.05$) from control were marked with a star. Control □ HS30′ ▨, HS60′ ⊡, HS72h ⊞, ANA3d ▢, ANA6d ■.

treatments produced higher amounts of ethanol than C. Surprisingly at 14 °C ($S2_0$ and $S3_0$), ethanol concentration in C fruits was significantly higher than treated fruits. An explanation for this finding could be that ethanol generally increases with ripening, and C fruits are more advancing in ripening than treated fruits. A similar effect was also observed by Chervin, Truet, and Speirs (1999), who compared ethanol evolution in pears stored at 20 °C after air and hypobaric treatments. The toxic effect of fermentation end-products could affect enzymatic reactions related to ripening (Peppelenbos & Oosterhaven, 1998).

In Fig. 4B, it is remarkable the decay of fruit submitted to ANA6d treatment and stored at 2 °C ($S2_4$ and $S3_4$), probably caused by the combination of low temperature and anoxic stress that provoke irreversible tissue damage. Kays (1997) explained that some factors, such as differences in the ability to metabolize the accumulated ethanol after returning back to aerobic conditions, or the extension of the damage produced during the ethanol synthesis could be responsible for the extent and magnitude of the injury associated with anaerobic stress. Ethanol concentration in fruits submitted to 4 days at 20 °C, regardless of the storage temperature, remained at low values. The probable causes of ethanol reduction could be: the reversibility of the synthetic pathway, due to pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) enzymes synthesis (Wills, McGlasson, Graham, & Joyce, 1998), or ethanol volatilisation and/or transformation into esters (Chervin et al., 1999).

Fig. 5A shows that fruits analysed immediately after anaerobic treatments (ANA3d and ANA6d) and treatments producing transient low-aerobic conditions

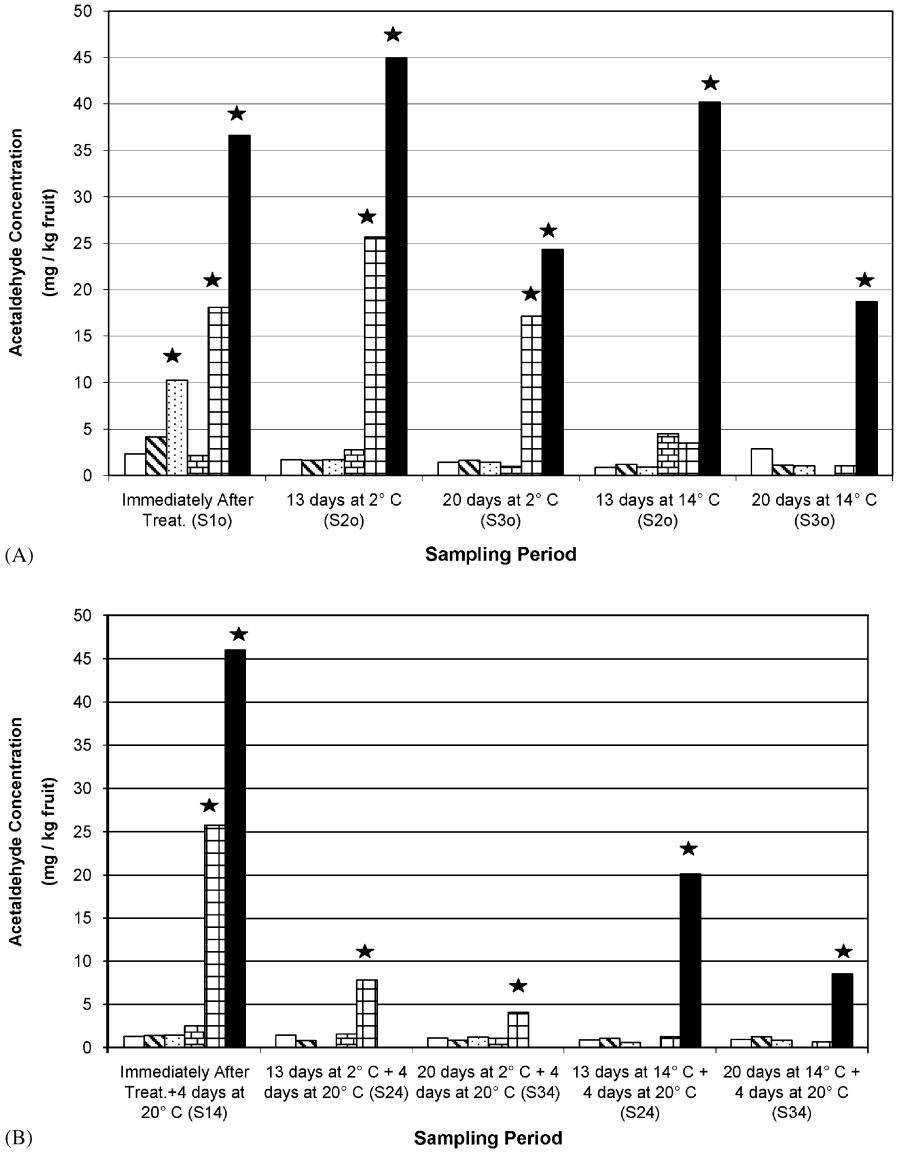

Fig. 5. Acetaldehyde concentration (mg/kg of fruit) in tomatoes untreated (Control), heated for 30 min (HS30′), 60 min (HS60′), or 72 h (HS72h), or subjected to anaerobiosis for 3 days (ANA3d) or 6 days (ANA6d). Fruits were analysed immediately after treatments or storage (A), or after 4 days at 20 °C (B). Treatments significantly different ($P<0.05$) from control were marked with a star. Control □ HS30′ ▧, HS60′ ▫, HS72h ▤, ANA3d ▢, ANA6d ■.

(HS60′) showed the highest AA concentration of the group. The magnitude of the increment would be a good indication of the extent of the treatment. After 13 and 20 days of storage at 2 °C, this parameter remained high in the fruit submitted to anaerobic conditions (ANA3d and ANA6d). The ethanol reduction during storage could be related to the AA increase observed after 13 days of storage at the same temperature (S2o). Ratanachinakorn et al. (1999) stated that an increment of ethanol induces the synthesis of ADH enzyme, the reversion of the reaction by a feedback mechanism, and consequently the transformation of the ethanol excess to AA. After 13 and 20 days of storage at 14 °C, only ANA6d treatment had a higher AA concentration than C.

Fig. 5B shows that after 13 and 20 days of storage at 2 °C, only ANA3d-fruits achieved a high AA concentration. After 13 and 20 days of storage at 14 °C, the highest acetaldehyde concentration was seen in the ANA6d treatment (Fig. 5A). A general view of Fig. 5B shows that AA concentration tended to decrease with time in fruits exposed to 20 °C, probably due to AA volatilisation induced by the temperature of storage.

Another research on the effect of stress treatments to prevent chilling injury in tomatoes fruits showed that storage at 2 °C produced some damage in treated and control fruits, though short heat treatments applied by water immersion were the most successful in controlling chilling injury. These treatments showed lower percentage of fruits with pitting or spoilage than control, while

both anaerobic and extended heat shock treatments induced higher levels of pitting than control (unpublished results).

4. Conclusions

The present work intends to depict the main physiological and biochemical events that take place during high temperature and anaerobic stress, comparing the application of these treatments in a same tomato variety. It was shown that anaerobic treatments and long-term heat shock treatments retarded colour development regardless of the storage temperature. The long-term heat treatments reduced TA, this reduction being mainly due to the decrease of malic acid. Anaerobic treatment induced a high ethanol accumulation but, after holding samples at 20 °C for 4 days, ethanol decreased in samples anaerobically treated for 3 days. An extension of anoxia treatment apparently produced irreversible metabolic changes. An increment in AA concentration was induced by anaerobic treatments and also by "low aerobic" conditions produced by water-heated tomatoes (HS60′). This "anaerobic effect" of a heat shock applied by water immersion should be considered when analysing this kind of treatment. Extended storage reduced AA concentration probably because of the high vapour pressure (low boiling temperature) of this compound.

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