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Evaluation of alternative preservation treatments (water heat treatment, ultrasounds, thermosonication and UV-C radiation) to improve safety and quality of whole tomato

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Abstract:	Previously optimized postharvest treatments were compared to conventional chlorinated water treatment in terms of their effects on the overall quality of tomato (cv. Zinac) during storage at 10 ºC. The treatments in question were; hot water treatment (WHT: 40 ºC, 30 min), ultrasounds (US: 45 kHz, 80%, 30 min), thermosonication (TS: 40 ºC, 30 min, 45 kHz, 80%) and ultraviolet irradiation (UV-C: 0.97 kJ.m-2). The quality factors evaluated were colour, texture, sensorial analysis, weight loss, antioxidant capacity, total phenolic content, peroxidase and pectin methylesterase enzymatic activities, and microbial load reduction. The results demonstrate that all treatments tested preserve tomato quality to some extent during storage at 10 ºC. WHT, TS and UV-C proved to be more efficient on minimizing colour and texture changes with the additional advantage of microbial load reduction, leading to a shelf-life extension when compared to control trials. However, at the end of storage, with exception of WHT samples, the antioxidant activity and phenolic content of treated samples was lower than for control samples. Moreover, sensorial results were well correlated with instrumental colour experimental data. This study presents alternative postharvest technologies that improve tomato (cv. Zinac) quality during shelf-life period, and minimize the negative impact of conventional chlorinated water on human safety, health and environment.

DETAILED RESPONSE TO REVIEWERS

Manuscript (FABT-D-14-00984R1) and authors

Evaluation of alternative preservation treatments (water heat treatment, ultrasounds, thermosonication and UV-C radiation) to improve safety and quality of whole tomato

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ANSWERS TO REVIEWERS COMMENTS

The answers are given just after reviewer's comments transcription.

Reviewer # 1

Fix grammatical errors in abstract section

Answer: Following reviewer's observations, and after requesting the help on a English colleague, the authors addressed the grammatical errors and reformulated the Abstract section:

-Lines 2-15: "The effect of different previously optimized postharvest preservation treatments: water heat treatment (WHT: 40 ºC, 30 min), ultrasounds (US: 45 kHz, 80%, 30 min), thermosonication (TS: 40 ºC, 30 min, 45 kHz, 80%) and ultraviolet radiation (UV-C: 0.97 $kJ.m^{-2}$), as alternative to conventional chlorinated-water treatment, on the overall quality of tomato (cv. Zinac) during storage at 10 ºC was evaluated. The evaluated quality factors were colour, texture, sensorial analysis, weight loss, antioxidant capacity, total phenolic content, peroxidase and pectin methylesterase enzymatic activities, and microbial load. Results demonstrated that all treatments conducted to preservation of tomato quality during storage at 10 ºC. WHT, TS and UV-C proved to be more efficient on minimizing colour and texture changes with the additional advantage of microbial load reduction, leading to a shelf-life extension compared to control fruits. However, at the end of storage, with exception of WHT samples, the antioxidant activity and phenolic content of treated samples was lower than for control samples. Moreover, sensorial results were well correlated with instrumental colour experimental data." **were changed to** "Previously optimized postharvest treatments were compared to conventional chlorinated water treatment in terms of their effects on the overall quality of tomato (cv. Zinac) during storage at 10 ºC. The treatments in question were; hot water treatment (WHT: 40 ºC, 30 min), ultrasounds (US: 45 kHz, 80%, 30 min), thermosonication (TS: 40 ºC, 30 min, 45 kHz,

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Reviewer # 2. Fix grammatical errors in main content of the paper (e.g ensure instead of insure).

Answer: Following reviewer's observations, the authors addressed the grammatical errors in the document:

-Lines 42: "insure" **was changed to** "ensure"

Evaluation of alternative preservation treatments (water heat treatment, ultrasounds, thermosonication and UV-C radiation) to improve safety and quality of whole tomato

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Abstract

Previously optimized postharvest treatments were compared to conventional chlorinated water treatment in terms of their effects on the overall quality of tomato (cv. Zinac) during storage at 10 ºC. The treatments in question were; hot water treatment (WHT: 40 ºC, 30 min), ultrasounds (US: 45 kHz, 80%, 30 min), thermosonication (TS: 40 ºC, 30 min, 45 kHz, 80%) and ultraviolet irradiation (UV-C: 0.97 kJ.m-2). The quality factors evaluated were colour, texture, sensorial analysis, weight loss, antioxidant capacity, total phenolic content, peroxidase and pectin methylesterase enzymatic activities, and microbial load reduction.

The results demonstrate that all treatments tested preserve tomato quality to some extent during storage at 10 ºC. WHT, TS and UV-C proved to be more efficient on minimizing colour and texture changes with the additional advantage of microbial load reduction, leading to a shelf-life extension when compared to control trials. However, at the end of storage, with exception of WHT samples, the antioxidant activity and phenolic content of treated samples was lower than for control samples. Moreover, sensorial results were well correlated with instrumental colour experimental data.

This study presents alternative postharvest technologies that improve tomato (cv. Zinac) quality during shelf-life period, and minimize the negative impact of conventional chlorinated water on human safety, health and environment.

Keywords: Water heat treatment; ultrasounds; thermosonication; ultraviolet radiation; tomato; postharvest quality.

Introduction

Tomato (*Solanum lycopersicum*) is a climacteric fruit with a rapid ripening and a short shelf-life, which present serious limitations for its efficient handling and transportation (Klee and Giovannon 2011). For these reasons, the optimization of the postharvest care of tomato fruits to obtain satisfactory quality is very important. If quality is maintained, market life can be extended, opening new opportunities and adding value to the fruit.

There have been numerous treatments applied to fresh tomato following harvest and prior to storage or marketing, in attempts to prolong storage life. These include various chemical, physical and biological treatments, including calcium applications (Wills et al. 1977), 1-methylcyclopropene (1-MCP) (Su and Glubber 2012), modified atmosphere (Mathooko 2003), edible coatings (Dávila-Aviña et al. 2011), and antagonists (Mari et al. 1996). While many of these treatments have shown some effectiveness in reducing decay, maintaining quality, and/or enhancing desirable characteristics, few have been widely used commercially given the process complexity and the high costs involved.

However, the most promising of the different emerging technologies to maintain the tomato quality appear to be water heat treatment (WHT) (Pinheiro et al. 2014; Pinheiro et al. 2012a), ultrasounds (US) (Pinheiro et al. 2012b), thermosonication (TS, combination of sonication and heat) (Pinheiro et al. 2012c) and ultraviolet radiation (UV-C) (Pinheiro et al. 2010). These treatments intend to eliminate unwanted bacterial load, ensure high nutritional and sensory quality, as well as extend its shelf life and storability. Moreover, these postharvest treatments were established to achieve other important requirements, such as minimizing environment hazards, addressing consumer-oriented concerns (health promoting compounds, food safety) and low treatment cost, which represent additional arguments to the widespread use of these technologies.

The aim of this study was to evaluate and compare the effects of postharvest treatments previously optimized: water heat treatment (WHT), ultrasounds (US), thermosonication (TS) and ultraviolet radiation (UV-C), on physical (colour, firmness, weight loss), biochemical (enzymatic activities: peroxidase and pectin methylesterase), nutritional (antioxidants and total phenolic content) and sensorial quality (colour and degree of deterioration rating), and microbial load (mesophylic and yeasts & moulds load) on tomato (cv. Zinac) fruits, during 30 days of storage at

10 ºC. Moreover, this study intends to select one treatment that contributes to delaying ripening deterioration changes, minimizing the microbial development while maintaining sensorial quality.

Materials and methods

Plant material

Tomato (*Solanum lycopersicum*, cv. 'Zinac') was obtained from a commercial greenhouse Carmo & Silvério in centre west of Portugal. Fruits were harvested at mature-green stage and their classification was performed through external colour, according to USDA standard tomato colour classification (USDA 1991). Tomatoes were selected with uniform colour and size, and without bruises or signs of infection.

Treatments

Tomato fruits were divided into five groups of *ca.* 15 kg each: washed with chlorinated water (HIPO – Ctr samples) and treated with alternative treatments: water heat treatment (WHT), singular ultrasounds (US), ultrasounds combined with heat (thermosonication, TS) and ultraviolet radiation (UV-C).

Chlorinated water treatment

A chlorinated water treatment (150 ppm at 5 ºC, pH 6.5 during 2 min) (Bartz et al. 2001) as decontamination methodology was used as control treatment during storage period at 10 ºC.

WHT, US and TS treatments

The WHT, US and TS treatments were carried out in a water bath (Elma Transsonic Cleaning baths – multiple-frequency units - Elma GmbH & Co, Singen, Germany) with 45 L capacity, and using previously optimized conditions. For the **WHT** tomato samples were subjected to a temperature of 40º ± 0.5 ºC during 30 min (Pinheiro et al. 2012a), for the **US** a constant US frequency of 45 kHz and power level of 80% during 30 min was applied on tomato samples (Pinheiro et al. 2012b), and for the **TS** a combination of US and WHT conditions were applied

(45 kHz, 80%, 40 ºC during 30 min) (Pinheiro et al. 2012c). After treatments, samples were cooled and paper dried for excess water removal.

UV-C treatment

The **UV-C** samples treatments were conducted on a closed box (43 cm (w) x 50 cm (l) x 24 cm (h)) equipped with two germicidal lamps (TUV 15W/G15 T8, Philips, Holland) emitting at 254 nm and placed 10 cm above tomato fruits. The box was covered with aluminium foil to promote a homogeneous light distribution. Prior to use, UV-C lamps were stabilized by turning them on for 15 min. Whole tomatoes were placed in a single layer on the illumination area at the fixed distance and rotated manually (180º) in order to ensure total UV exposure. The tested UV-C intensity was measured by a photo-radiometer (DELTA OHM LP9021 UVC, Padova, Italy), giving corresponding doses of 0.97 $kJ.m^{-2}$ after exposure time of 3 min, as previously optimized by Pinheiro et al. (2010).

A physical-chemical and microbial characterization of tomato fruits after washing with chlorinated water (Ctr samples) and immediately after treatments (WHT, US, TS and UV-C) was performed to provide a baseline comparison to the treatments effects.

After treatments, all samples: control (Ctr) and treated (WHT, US, TS and UV-C) were stored at appropriate storage temperature, as previously optimized by Pinheiro et al. (2013): 10º ± 0.5 ºC and 90 ± 1 % RH (S600 Pharma, Fitoclima, Aralab – Equipamentos de Laboratório e Electromecânica Geral Lda., Portugal) during 30 days. Temperature and humidity were monitored with a HygroLog data logger (Rotronic AG, Bassersdorf, Switzerland).

Methods

Colour

Tomato colour was evaluated using a tristimulus colorimeter (Minolta chroma Meter, CR-300, Osaka, Japan). The instrument was calibrated using a white standard tile (L*=97.10, a*=0.19, b*=1.95), using the illuminate C (10^{th} observer). A CIE colour space co-ordinates, L*a*b* values, was determined. L* values represent the luminosity of samples (0-black to 100-white), a* and b* values indicate the variation of greenness to redness (-60 to +60) and blueness to yellowness (-

60 to +60), respectively. From the coordinates CIELab, hue value (°*h*=arctg (b*/a*)) was calculated. Sixteen measurements were taken from 4 fruits for each treatment.

Texture

Texture was determined by a penetration test with a Texture Analyzer (TA.HDi, Stable Microsystem Ltd, Godalming, UK), using a 50 N load cell and a stainless steel cylinder probe with a 2 mm diameter. The penetration test was performed at 3 $mm.s^{-1}$ of speed and at 7.5 mm of penetration distance. Force-distance curves were recorded and firmness (maximum peak force, N) was used as indicator of texture parameter. Firmness was measured after holding the tomato at room temperature for 2 hours, to avoid storage temperature effects on determination. Sixteen measurements were taken from 4 fruits for each treatment.

Sensorial analysis

Sensorial analysis was performed using an analytical-descriptive test to discriminate the sensory quality attributes of Ctr and treated samples along refrigerated storage. A panel of 8 – 10 trained judges (members of UEISTSA), who met the basic requirements of sensory sensitivity according to ISO 8586- 1 (1993), in adequate conditions compliant to ISO 13299 (1995), identified and distinguished the sensory attributes of colour, global acceptability and deterioration index (visual evaluation) of fresh tomato fruits, using numeric rating scales as follows:

Colour rating system: 1 = green (0% red); 2 = breaker (<10% red); 3 = turning (10% < red < 30%); 4 = pink (30% < red < 60%); 5 = red (60% < red < 90%) and 6 = red (> 90% red).

Global acceptability rating system: 1 = highly acceptable; 2 = moderately acceptable; 3 = medium acceptable (consumer limit); 4 = moderately unacceptable; 5 = unacceptable.

Degree of deterioration rating system: 0 = absent; 1 = very slight; 2 = moderate; 3 = severe.

Moderate to severe deteriorated fruits are not commercialized and represent disorders affecting from 25% to 50% (anchor 2) and over 50% of fruits surface (anchor 3), respectively.

Panellists were asked to evaluate samples during storage, scoring the level of difference in perceived intensity between treated and Ctr samples with respect to each attribute.

Weight loss

Weight loss was measured by Van Dijk et al. (2006) method. A batch constituted by three fruits per treatment was weighted. After weighting, tomato was put back to original storage conditions. The weight loss was calculated relative to the weight at day zero (t=0).

$$(\%)\ Weight\ \ loss = \frac{W_0 - W_t}{W_0} \text{ x } 100 \quad (1)$$

where W_0 is the average weight of the first batch at day 0 and W_t is the average weight of the same batch at day t. Two replicates were carried out.

This parameter could be used to define fruit quality, due to its impact on the tissue which becomes dull and very soft when weight loss is high.

Antioxidant activity

Antioxidant activity was performed by using ABTS method as described by Re et al. (1999). This stable radical cation was performed by mixing 10 mL of a 7 μmol/L ABTS solution with 10 mL of a 2.45 μmol/L $K_2S_2O_8$ solution. After 24 h at room temperature in the darkness, the ABTS stock solution was ready to use. An ABTS working solution was prepared daily by diluting the ABTS stock solution with ethanol to an absorbance between 0.680-0.720 at 734 nm in an ATI Unicam UV/VIS UV4 spectrophotometer (Unicom Limited, Cambridge, United Kingdom). Thirty microliters of extract were mixed with 3 mL of ABTS working solution during 7 min at 30 ºC. Trolox solutions (150–2500 μmol/L) were used for constructing a regression line. Results were expressed as μmol Trolox equivalents of antioxidant capacity (μmol TEAC.100 g^{-1}) of fresh fruits. Six measurements for each sample were obtained.

Total phenolic content

Total phenolic were determined using the Folin-Ciocalteau reagent (Singleton and Rossi 1965). Five tomato fruits were chopped and blended for 2 min until uniform size. Samples (10 g) were homogenized in 70% aqueous methanol (10 ml), using a Yellow line DI 25 basic polytron (IKA-Labortechnik, Stauten, Germany), centrifuged (Sorvall RC-5, rotor SS34, DuPont, Wilmington, United States) at 19000 rpm for 20 min at 4 ºC, and the supernatant was collected. One hundred microliter of supernatant was mixed with 5 ml of Folin-Ciocalteau (1/10, v/v) and 4 ml of

Na_2CO_3 (7.5%, w/v). The mixture was placed in a water-bath (45 °C for 15 min) and the absorbance measured at 765 nm in an ATI Unicam UV/VIS UV4 spectrophotometer (Unicom Limited, Cambridge, United Kingdom), using gallic acid as a standard. Results were expressed as milligram gallic acid equivalents (mg GAE.100 g^{-1}) of fruit weight. Six measurements for each sample were carried out.

Enzymatic activity

Pectin methylesterase (PME) activity was determined by a spectrophotometric procedure optimized by Pinheiro et al. (2012d). Four tomato fruits were chopped and blended for 2 or 3 min. Ten g of blended tomato were homogenized with 90 ml of cold 1.5 M NaCl, using a Yellow line DI 25 basic polytron (IKA-Labortechnik, Stauten, Germany). The mixture was centrifuged (Sorvall RC-5, rotor SS34, Dupont, Wilmington, United States) at 19000 rpm for 20 min at 4 °C. Then the supernatants were filtered and adjusted at desired pH (CrisonMicro pH 2001, Crison Instruments, Barcelona, Spain) with NaOH 0.1 and 0.01 N. The standard reaction mixture contained 0.5% pectin from citrus fruits (Sigma Aldrich - 2 ml), 0.01% cresol red in sodium phosphate buffer at 0.003 M and pH 8.8 (0.150 ml), enzyme extract and water (0.05 and 0.800 ml, respectively). The reaction mixture was incubated at 30°C and the activity was measured at 573 nm during 1 min (ATI Unicam UV/VIS UV4 spectrophotometer, Unicom Ltd., Cambridge, United Kingdom).

Peroxidase (POD) activity was determined as described in Yahia et al. (2007) with some modifications. Four tomato fruits were chopped and blended for 2 or 3 min. Ten g of blended tomato were homogenized with 50 ml sodium phosphate buffer (0.05 M pH 7.0) and centrifuged (Sorvall RC-5, rotor SS34, Dupont, Wilmington, United States) at 19000 rpm for 20 min at 4 °C. The reaction medium contained 2.855 ml sodium phosphate buffer (0.05 M, pH 6.0), 45 µl guaiacol (1%), 40 µl H_2O_2 (0.3%), and 60 µl enzyme extract. The absorbance was recorded at 470 nm with a Unicam UV-VIS spectrophotometer (Unicom Limited, Cambridge, United Kingdom).

Both enzymatic activities (POD and PME) were expressed as activity units per 100 g of fresh tomato, and the results were normalized in regard to the corresponding fresh tomato activity (% - P/P0*100, where P0 is the initial enzymatic activity at initial time and P the activity at time t). Final enzyme activities were determined from the average of four independent measurements from 4 fruits each.

Microbial count

Total mesophylic counts were performed according to ISO 4833 (2003). Ten g of sample were mixed with 90 ml peptone saline solution in a sterile stomacher bag and homogenized for 1 min using a Stomacher. Dilutions were made in peptone water as needed for plating. Plate Count Agar was used as the media for total mesophylic counts and incubated at 30 ºC for 3 days. Yeast and mould (Y&M) were determined according to NP 3277 (1987), using Rose Bengal Chloramphenicol Agar, surface inoculation and incubated at 25 ºC during 5 days. A total of three independent measurements were taken per sample and results were expressed as Log_{10} $cfu.g^{-1}$.

Data analysis

Data were subjected to analysis of variance (two-way ANOVA) using Satistica v.7.0 (Statsoft, 2004) to assess treatments and storage period effects on tomato quality. Tukey test was used to determine significant differences between samples ($P < 0.05$).

Results and discussion

Colour

Colour is probably one of the most important attributes influencing tomato overall quality, as it affects consumer perception and acceptance. A number of physical-chemical changes occur when tomato progress from the mature-green to red maturity stage. The most obvious change is the external colour, which is associated with chlorophyll loss and lycopene accumulation due to ripening (Saltveit 2005).

Only the luminosity did not differ significantly ($P > 0.05$) after treatments (*ca.* 48-49). Treatments with heat application (WHT and TS) lead to a significant ($P < 0.05$) loss of tomato green colour (increase of a* value) in comparison with control samples (-13.1 ± 1.0). On the other hand, the colour of US and UV-C samples was similar ($P > 0.05$) to control samples, therefore did not affect the initial tomato colour characteristics.

Fig. 1A presents the behaviour of tomato L* parameter during storage at 10 ºC. As expected, during storage L* values decrease, reflecting an increase of tomato darkening due to carotenoid synthesis (Yahia et al. 2007). Only on UV-C samples an increase of L* value was observed. However, only in Ctr samples a marked reduction of L* value was observed from day 4th to 20th, reaching the lowest value of all samples (L* = 42.3). This fact indicates that all treatments lead to a delay of tomato colour changes.

The impact of treatments on greenness (lower a* value) and redness (higher a* value) of tomato fruit can be observed in Fig. 1B. The Ctr sample showed a fast red colour development (higher a*) compared with treated samples (WHT, US, TS and UV-C). Moreover, from days 8 till 22 the a* value of Ctr samples reached a maximum of 16.6. Similar behaviour was reported by Guillén et al. (2006) on different tomato cultivars ('Cherry' and 'Daniela', round; 'Patrona', pear-type; 'Raf', lobular) and two maturity stages (S1 and S2), during 28 days storage at 10 ºC.

The US and WHT samples showed a pronounced increase of a* value from day 16 until the end of storage, attaining 7.6 and 0.09, respectively. The TS treatment, that combines the synergistic effect of heat and US, showed the same beneficial effect as UV-C radiation, and both treatments delay red colour development and exhibited the lowest final a* value (-7.8).

The study developed by Alexandre et al. (2011) in red bell pepper demonstrated that thermosonication at 50 ºC_35 kHz_2 min retained sample colour better than US treatments. Vicente et al. (2005) observed also that peppers treated with UV-C radiation had higher hue values than Ctr samples stored at 10 ºC.

Texture

Firmness is another important quality-related attribute in tomato fruits, and may be considered as a final quality index by which the consumer decides to purchase the product. The major problem concerning tomato firmness is related to tissue softening which usually involves one of

two mechanisms: weight loss with turgor loss, or a result of enzymatic activity leading to structural changes in the principal cell wall components (cellulose, hemicellulose and pectin) (Alia-Tejacal et al. 2007). Therefore, any treatment able to delay fruits softening is potentially helpful to extend postharvest shelf life and maintain product quality.

After washing with chlorinated water tomato have an initial firmness of 12.5 ± 1.2. Pinheiro et al. (2013) reported a similar value (12.8 ± 0.3 N) for the same tomato cultivar and maturity stage.

Immediately after US, WHT, TS and UV-C treatments, no significant (P > 0.05) firmness changes were observed, and only a slight increase of *ca.* 6% was observed after US, TS and UV-C, while WHT samples firmness remained unchanged, compared to control.

Abreu et al. (2011) found a firming effect in *Rocha* pear after treatment at 35 ºC during 20 min. Possible reasons for firmness increase after heat treatment can be due to the activation of pectin methylesterase (PME) and subsequent formation of calcium pectates, or the heat changes effects on protein metabolism by suspending the synthesis of housekeeping proteins to produce heat-shock proteins (Brodl 1989).

Changes in stored tomato firmness at 10 ºC are shown in Fig. 2. It is evident that all samples present a gradual and pronounced softening along the first 8 storage days. The firmness reduction was more pronounced for Ctr and US samples, *ca.* 31% and 26%, respectively, compared to initial value. After the 16th day, no statistically significant differences (P > 0.05) in firmness of Ctr, US and TS were found. Moreover, the firmness of UV-C samples did not change from day 8. Both samples of UV-C and WHT presented the highest maximum force at the end of storage (more 30% and 26%, respectively, comparing with the Ctr), revealing an advantage of these technologies for delaying tomato softening during postharvest.

Previous works have been reporting the benefits of UV-C treatment to delay fruits ripening and maintain postharvest firmness, such as in Kent and Seascape cultivars of strawberry treated with UV-C doses of 1 and 4.1 kJm^{-2}, respectively (Barka et al. 1999; Pan et al. 2004). Barka et al. (2000) suggested that delay of softening on UV-C tomato would be due to lower cell wall degradation, and cell wall degrading enzymes may be targets of UV-C radiation.

Sensorial analysis

Fig. 3 presents sensorial results of colour, global acceptability and deterioration index of Ctr and treated tomato samples, immediately after treatment and during storage.

Immediately after treatments, no significant differences ($P > 0.05$) were detected in fruit colour perception between Ctr and treated samples, indicating good acceptability (score 1) and undetectable deterioration (score 0). Comparing sensorial analysis with previous experimental colour parameter (a* value) a good and significant correlation ($R^2 = 0.93$, $p = 0.00$) was obtained, as shown in Fig. 3 (D).

From day 8 of storage, the Ctr samples were the less preferred in terms of global acceptability (Fig. 3), due to firmness and weight loss changes. Also, all treated samples were characterized by a trained panel as better fruits during storage. The UV-C and TS-treated samples presented the lowest score (1.9 = moderately acceptable), revealing the consumer preference at the end of storage. The global acceptability behaviour correlates well with fruits colour ($R^2 = 0.95$, $p = 0.00$).

Regarding deterioration index (Fig. 3C), until the first four storage days no significant difference ($P > 0.05$) was detected in all samples. After this period, the Ctr samples started to be negatively rated, becoming very slight to moderate (25-50%), defined as consumer limit.

Weight loss

Tomato weight loss is affected by several pre and postharvest factors, such as harvest date and storage temperature (Alia-Tejacal et al. 2007).

The influence of treatments and storage period on tomato weight loss is shown in Fig. 4. All treatments present an increase of tomato weight loss during storage at 10 ºC. The rate of weight loss per day of control sample was 0.20% and differs significantly ($P < 0.05$) from other samples. Treatments of US and TS have rate values per day of 0.17%, 0.14%, respectively, and both WHT and UV-C samples a rate of 0.15%.

The increase of weight loss observed on WHT and TS was lower than observed in other samples. This increase might be a response to the higher temperature and long time treatment that increase the vapour pressure deficit and the water loss of fruit (Assi 2004). However, treatments such as UV-C, WHT and TS lead to the lowest value of WL (4.4%, $P > 0.05$) at the end of storage. The low value of weight loss observed for WHT samples during storage period has benefic effects mainly on fruit's appearance. Opposite results were obtained by Forney

(1995), that after testing different heat treatment conditions (25-52 ºC, 1-40 min) on broccoli, a higher rate of average weight loss per day (*ca.* 6% than observed in Ctr sample (4.6%)) was observed. In strawberries, hot air treatment at 45 ºC during 3 h conducted to an increase of weight loss (more 1% than in untreated fruits) (Vicente et al. 2002).

With the exception of Ctr sample, US treatment was the least effective on reducing tomato weight loss, reaching a value of 5% at the end of storage.

Along 30 days of storage at 10 ºC no sample reached to the criteria defined by Pal et al. (1997) and Acedo (1997) (In Getinet at al. 2008) of about 10% of weight loss, which confirms that this index is not a good quality criteria for tomato 'Zinac' (Pinheiro et al. 2013).

Antioxidant activity

Natural antioxidants, present in fruits and vegetables, have gained increasing interest mostly by consumers due to epidemiological results indicating that high consumption of natural antioxidants is associated with lower risk of cardiovascular disease and cancer (Temple 2000). Tomato is considered a high nutritious fruit because it contains a considerable level of vitamins A, E (tocopherols) and C, lycopene, β-carotene (precursor of vitamin A in the human body), fibres, and phenolic compounds namely flavonoids and phenolic acids (Soto-Zamora et al. 2005).

Tomato antioxidants (AO) content is influenced by cultivar, maturity stage and applied analytical methodology (Cano et al. 2003; Raffo et al. 2002). In our study, an initial value of 599.4 ± 29.4 μmol Trolox.100g^{-1} was determined for Zinac cultivar of mature-green tomato. Lower values of antioxidant activity on cherry tomato (''Pomodoro di Pachino'', cv. Naomi F1) were found by Raffo et al. (2006) at different harvesting time of the year (< 420 μmol Trolox.100g^{-1}).

Only the AO content of TS samples (518.2 μmol Trolox.100g^{-1}) differ significantly ($P < 0.05$) immediately after treatment. However, no significant differences between treatments were observed ($P < 0.05$). Tomato AO as affected by treatment during storage period at 10 ºC is shown in Fig. 5. Overall, after a decline during the first 4 days of storage, an increase of AO was observed until the end of storage: 16, 37, 30, 61 and 80 % on TS, UV-C, US, Ctr and WHT, respectively. In our study, the intensity of UV-C applied (0.97 kJ.m^{-2}) promoted a lower AO

value (15%) than reported by Chang-hong et al. (2012), where UV-C treatment at 2 kJ.m^{-2} induced an increase of 53% after 21 storage days.

Total phenolic content

Phenolic compounds are one of the major contributors to the antioxidant properties of fresh products (Gil et al. 2002), and are associated with anti-mutagenic and anticancer properties as well as with reduced cardiovascular diseases (Dillard and German 2000).

Untreated tomato showed an initial value of total phenolics (TPC) of 30.1 ± 1.9 mg GAE.100g^{-1}. The initial content of TPC of cv. Zinac tomato was higher than observed by Georgé et al. (2011) on red tomato (16.0 mg GAE.100 g^{-1}) and Brat et al. (2006) (9.8–23.0 mg GAE.100 g^{-1}). The differences between initial content of TPC could be due to maturity stage, cultivar or analytical methodology used. Moreover, Macheix et al. (1990) noted that genetic control can be responsible by phenolics accumulation in vegetable food, and the external factors such as UV radiation may also have a significant effect on this parameter.

Immediately after treatment, and compared to Ctr samples, a TPC increase of 14% on US samples and reductions of 14% and 12% on WHT and TS samples, respectively, were observed. The reduction of TPC in WHT samples can be due to phenylalanine ammonia lyase (PAL) activity reduction. The application of heat treatments interrupts normal protein synthesis and reduces PAL accumulation and phenolic synthesis (Vicente et al. 2002). Opposite effects were observed after US treatment, since an improvement of normal protein synthesis was attained with this methodology.

The TPC content was lower for treated samples than control fruits, during almost all storage time. After 8 days of storage (Fig. 6) it is evident a maximum peak of TPC on Ctr samples (41.2 mg GAE.100g^{-1}), followed by a decline until the end of storage. This behaviour has been also observed for antioxidant capacity (Fig. 5).

At the end of storage, a similar (P > 0.05) increase of TPC was detected on UV-C and TS treatments, reaching values of 39.4 and 35.7 mg GAE.100g^{-1}, respectively. Heat treatment and UV-C radiation have been associated with enhancement of bioactive compounds, such as vitamins, carotenoids and phenolic compounds in fruits and vegetables (Heredia and Cisneros-Zevallos 2009).

On the other hand, these treatments may also influence the phenylpropanoid pathway responsible for phenolic synthesis (Ke and Saltveit 1989).

Enzymatic activity

It is known that pectin methylesterase (PME: EC 3.1.1.11) is correlated with texture changes of fruits and vegetables. Normally, the faster metabolic rate occurring during fruits ripening results in greater solubilisation of pectin (Morais et al. 2008) and consequently increases PME enzymatic activity.

A significant ($P < 0.05$) decrease of PME activity, ca. 12.5% on US & UV-C samples and 25% on WHT & TS samples, was observed, compared to Ctr sample. Similar PME reduction was reported by Pombo et al. (2009) after processing strawberry with a higher level of UV-C (4.1 $kJ.m^{-2}$).

Fig. 7A represents PME activity changes for control and treated tomato samples during storage at 10 ºC. In control samples, PME activity increased gradually *ca.* 25% until day 16^{th} and then a slight decrease was observed. Previous works reported similar behaviour for PME activity, mostly in tropical fruits like sapodilla, guava, papaya and carambola (Morais et al. 2008; Miranda et al. 2002; Abu-Goukh and Bashir 2003; Ali et al. 2004).

During storage, PME activity levels were significantly ($P < 0.05$) lowest in WHT, US and UV-C compared to control and TS samples. Similar ($P > 0.05$) PME activities were observed along storage period for WHT and US samples. The enzymatic activity found on UV-C, TS and WHT samples was consistent with firmness value, where lower PME activity leads to higher fruits firmness.

The benefic effect observed with TS treatment on the decrease of PME activity was also observed by Siwach (2012), where after processing mosambi juice with thermosonication at 80 °C for 20 min (50 kHz, 400 W) a PME inactivation of 96.8% was obtained.

Quality changes related to enzymatic activity are also due to peroxidase (POD), which is responsible for several biochemical reactions, such as oxidation of many organic compounds, leading to product's flavour, colour and nutritional degradation (Thongsook and Barrett 2005).

A significant increase ($P < 0.05$) of POD activity, *ca.* 56, 26, e 22%, was observed immediately after WHT, TS and UV-C, respectively, with exception of US. The increase of enzymatic activity was due to response of different types of stresses, both biotic and abiotic (Rivero et al. 2001).

Fig. 7B presents POD enzymatic activity of control and treated tomato samples as a function of storage at 10 ºC. There is a reduction of POD activity in all samples during storage, being more pronounced until 8th storage day on Ctr and TS samples, remaining constant until day 30. At the end of storage the WHT samples reach a value of 83%, followed by TS (49%), UV-C (34%), Ctr (26%), and US (22%).

Microbial load

Initial mesophilic counts of 5.2 ± 0.2 Log_{10} and 3.2 ± 0.1 Log_{10} of Y&M were found in fresh tomato at arrival to laboratory. After treatments a reduction in all samples was detected. The conventional decontamination treatment (Ctr) provided a decrease of mesophyles and Y&M, *ca.* 1.6 and 1.2 Log_{10} cycles, respectively, and is consistent with results obtained by Pinheiro et al. (2014) and Felkey et al (2006), where the maximum of microbial reduction achieved was less than 2.0 Log_{10}. In this study the reduction of initial microbial load of tomato fruits as a result of UV-C radiation (0.97 $kJ.m^{-2}$) was more efficient than previously obtained (Pinheiro et al. 2010), and this decrease maybe be attributed to a higher microbial load presented on the raw material. The germicidal effect of UV-C radiation can be attributed to photochemical lesion induced on microorganisms DNA and RNA, resulting in the inability to carry out normal transcription and replication of nucleic acids and normally the cell dies (Bolton and Linden 2003; Unluturk et al. 2008).

TS treatment contributed to the highest decrease on mesophylic counts, immediately after UV-C radiation. This efficiency can result from the combination of physical and chemical mechanisms which occur during cavitation as reported by Adekunte et al. (2010), i.e. the formation of pores outside the cell membrane, disruption of cell structures and breakage of cells. On the other hand, singular effect of US did not conduct to an effective reduction on mesophylic count on whole tomato fruits.

The mesophylic and Y&M of control and treated tomato fruits as affected by treatment and storage at 10 ºC are shown in Table 1.

Overall, during storage a growth of mesophyles and Y&M load in all samples was observed, and in the first 8 days of storage the microbial load increased at a faster rate. According to the maximum value recommended (aerobic mesophilic flora < 5.1 Log_{10} $cfu.g^{-1}$) by the International Commission on Microbiological Specifications for Foods (ICMSF 1986), the TS and UV-C treated samples did not reach to this limit, during the study. So, a gain of 26 storage days at 10 ºC was achieved in these samples, when compared with the control (Ctr).

Conclusions

The present study demonstrates that preservation technologies, when applied immediately after harvest, can ensure better fruit quality during postharvest period. The sensorial perception of colour and fruits firmness was not affected just after application of all treatments. At the end of storage, a better preservation content of TP, firmness and lower value of WL was achieved on UV-C treated samples, and the microbial load was reduced significantly, when compared with standard chlorinated water solution. Therefore, UV-C treatment appears to be an effective, environmentally safe method, that reduces water consumption and decay of tomatoes quality. This postharvest treatment may be considered for industrial use as potential tool to deliver healthier whole tomato fruits and prolong its storage period, reducing fruit quality losses.

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Figure 1
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Figure 1

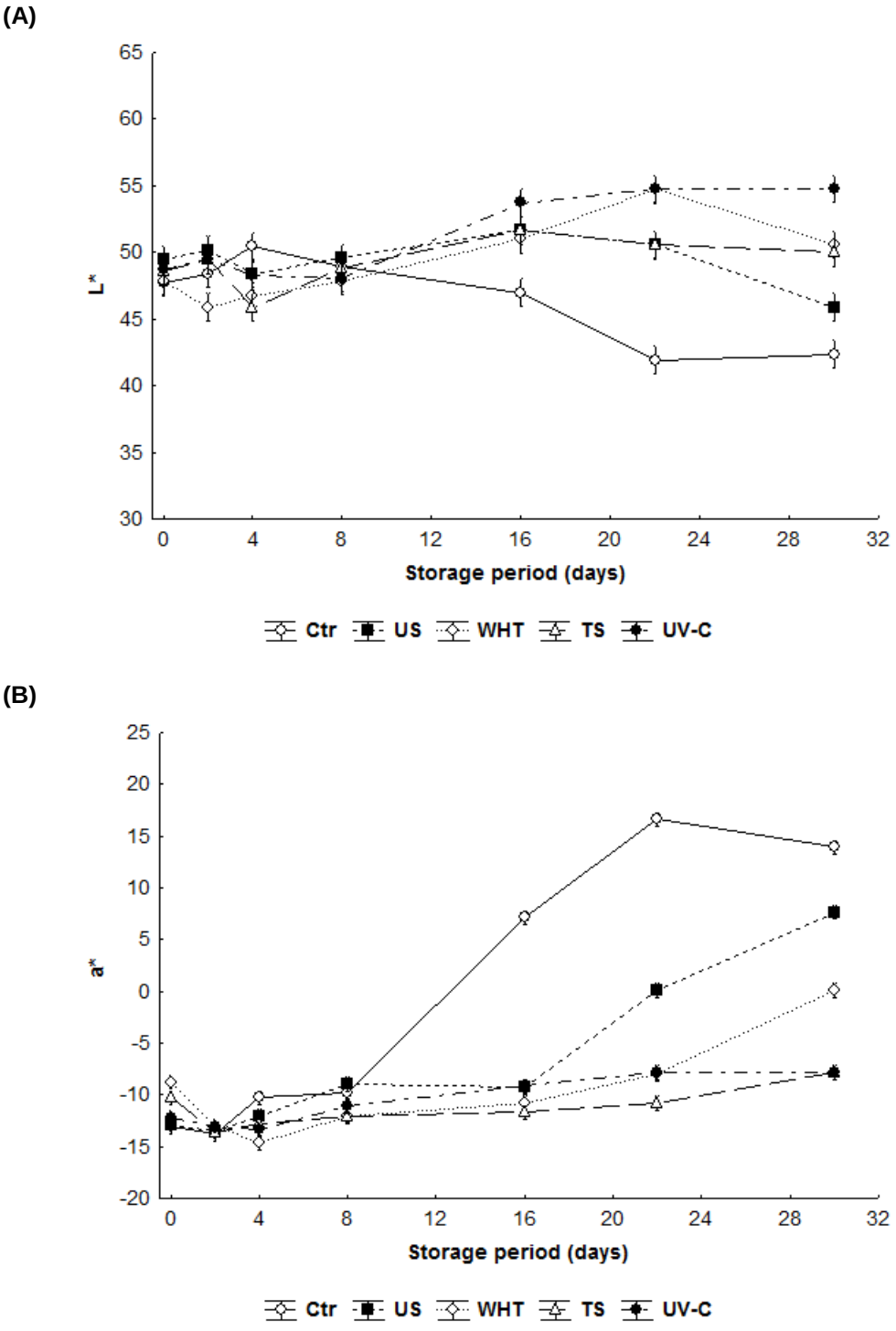

Fig. 1 – Changes in L* (A) and a* (B) colour parameters of control (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Vertical bars represent 95% of confidence intervals.

Figure 2
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Figure 2

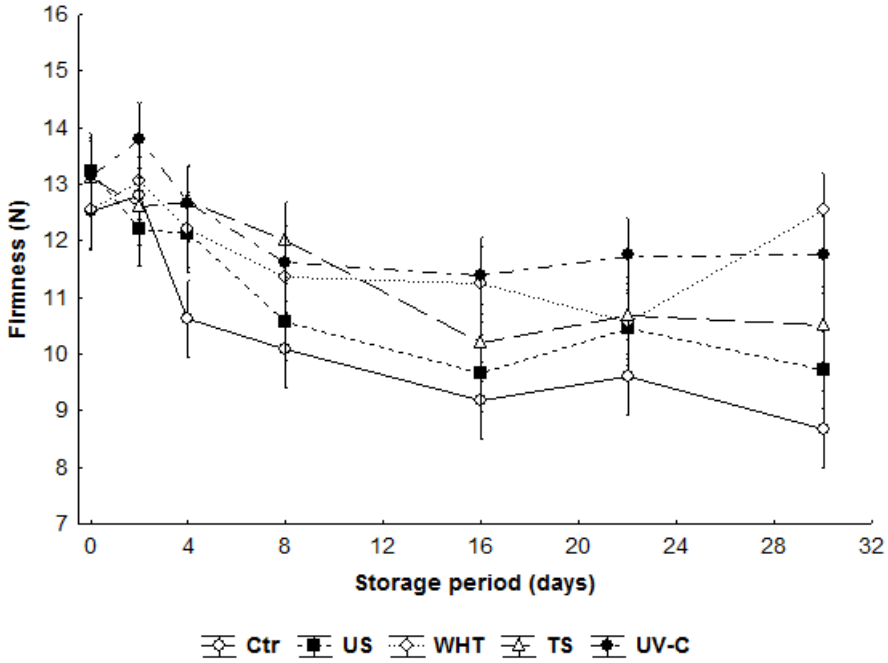

Fig. 2 – Changes in firmness (N) of control (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Vertical bars represent 95% of confidence intervals.

Figure 3
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Figure 3

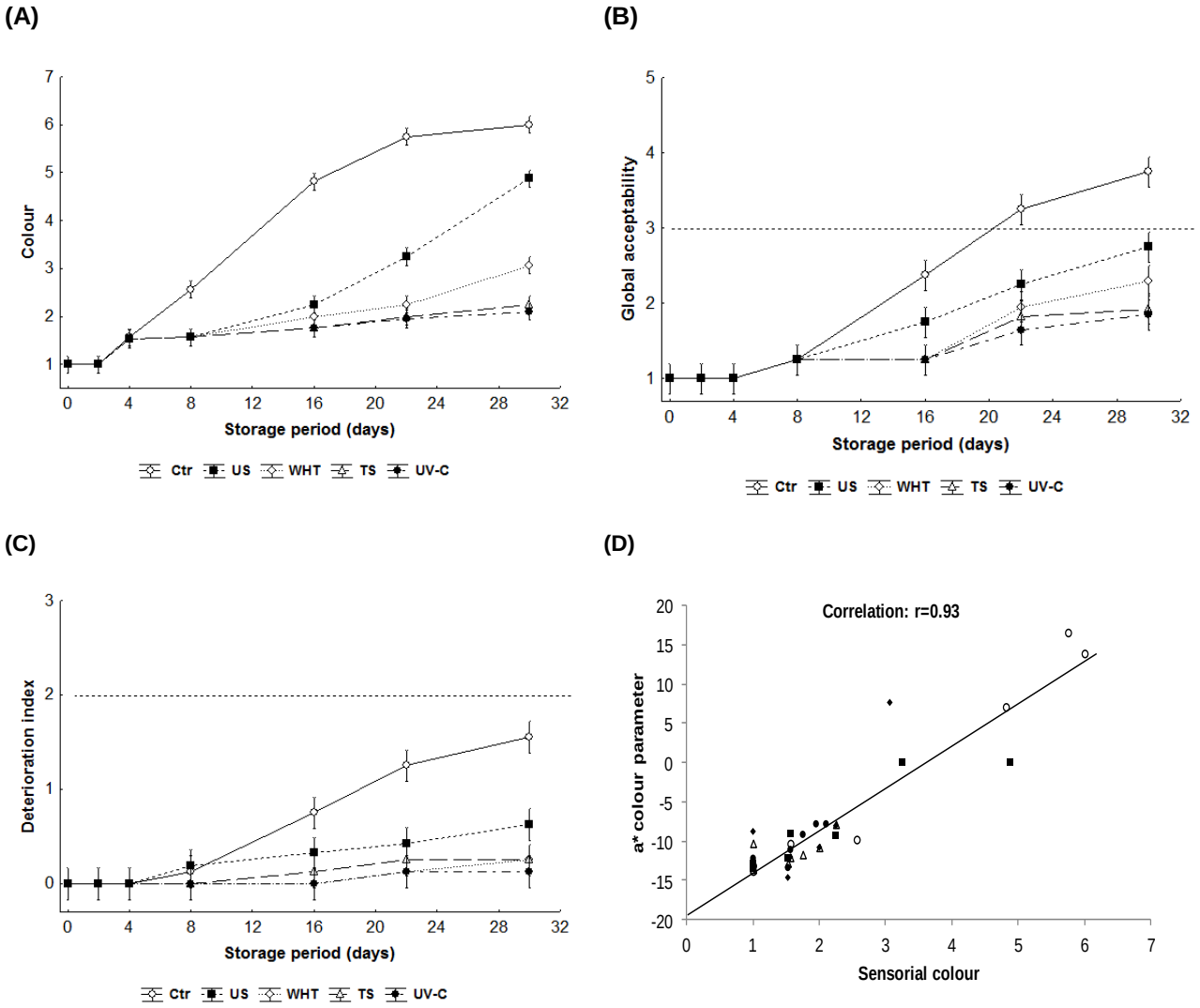

Fig. 3 – Changes in sensorial colour (A), global acceptability (B), and deterioration index (C) of (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Graph of correlation between sensorial colour and objective colour (D). Vertical bars represent 95% of confidence intervals.

Figure 4
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Figure 4

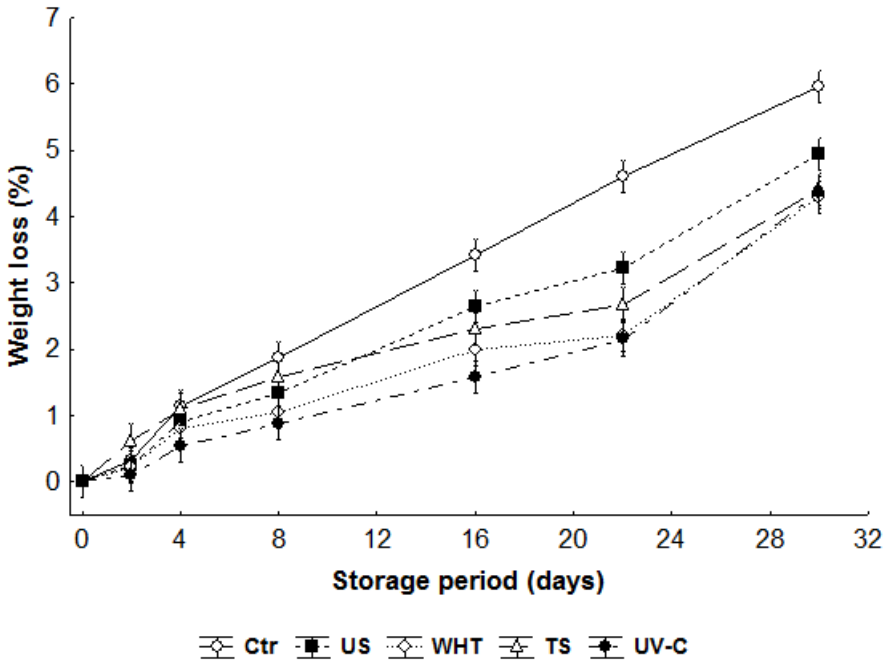

Fig. 4 – Changes in weight loss (%) of control (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Vertical bars represent 95% of confidence intervals.

Figure 5
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Figure 5

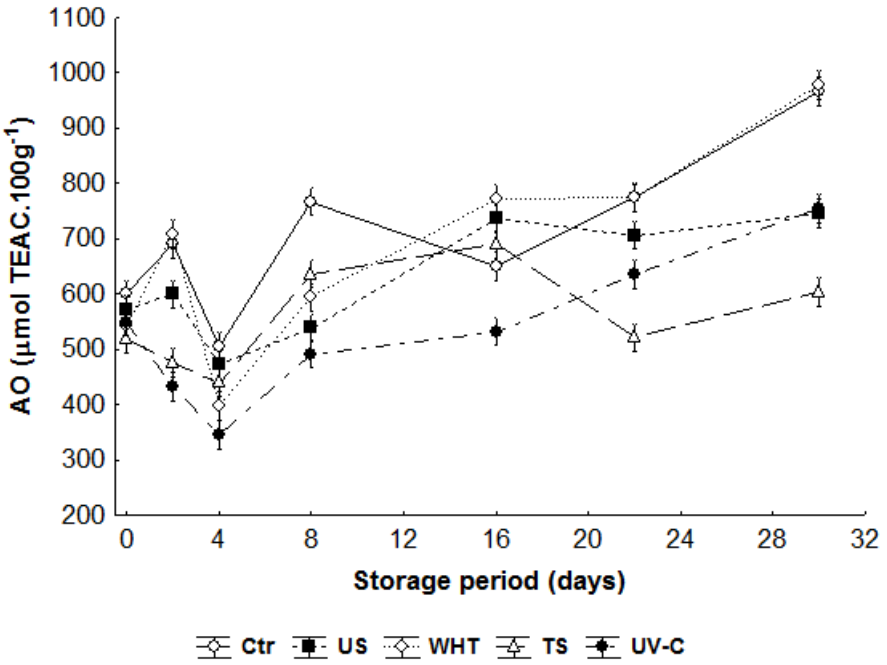

Fig. 5 – Changes in antioxidant activity (µmol TEAC.100g^{-1}) of control (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Vertical bars represent 95% of confidence intervals.

Figure 6
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Figure 6

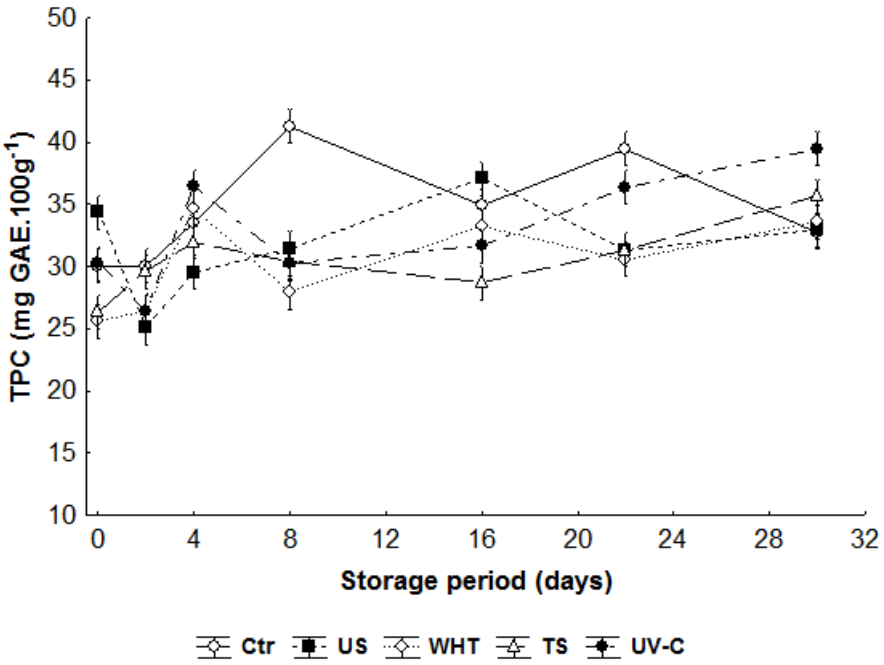

Fig. 6 – Changes in total phenolic content (TPC, mg GAE.100g^{-1}) of control (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Vertical bars represent 95% of confidence intervals.

Figure 7
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Figure 7

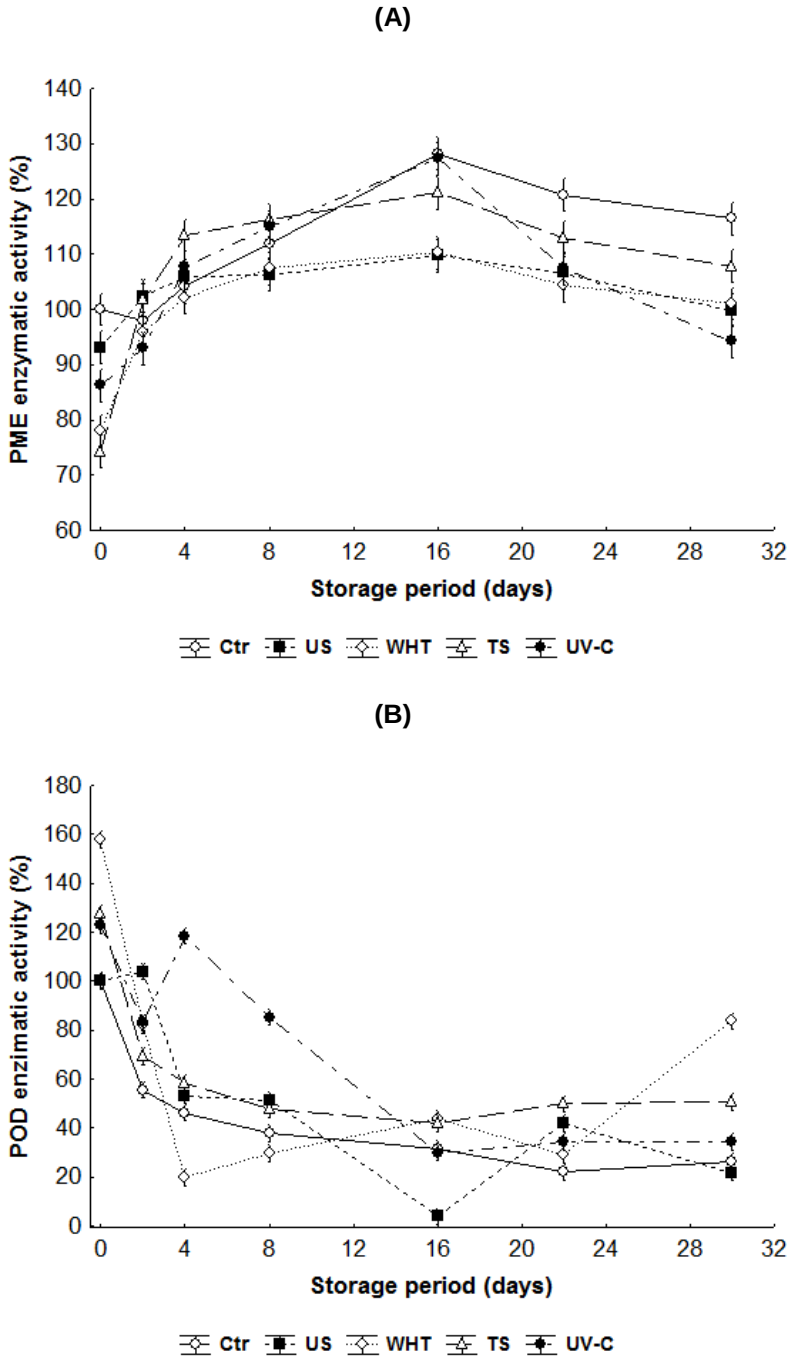

Fig. 7 – Changes in enzymatic activity of pectin methylesterase (PME, %) (A) and peroxidase (POD, %) (B) of control (Ctr) and treated (US, WHT, TS, UV-C) stored tomato at 10 ºC. Vertical bars represent 95% of confidence intervals.

Table 1
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Table 1

Table 1 – Microbial load: mesophyles and yeasts and moulds (average ± standard deviation) of control (Ctr) and treated (US, WHT, TS and UV-C) tomato during 30 days of storage at 10 ºC.

Samples	Storage period (days)	Mesophyles (Log_{10} (cfu.g^{-1}))	Y&M (Log_{10} (cfu.g^{-1}))
Ctr	**0**	$3.7^{k,l,m} \pm 0.1$	$1.9^{h,i} \pm 0.1$
	2	$3.7^{k,l,m} \pm 0.1$	$2.6^{l,m} \pm 0.1$
	4	$4.6^{n,o} \pm 0.4$	$3.2^{n} \pm 0.1$
	8	$5.9^{q,r} \pm 0.1$	$3.7^{o} \pm 0.1$
	16	$6.8^{s,t} \pm 0.4$	$4.0^{o} \pm 0.1$
	22	$7.2^{t} \pm 0.1$	$4.9^{p} \pm 0.1$
	30	$7.1^{t} \pm 0.1$	$5.1^{p} \pm 0.0$
US	**0**	$3.0^{j,k} \pm 0.1$	$1.5^{c,d} \pm 0.1$
	2	$2.8^{h,l,j,k} \pm 0.1$	$2.2^{f,g} \pm 0.2$
	4	$3.2^{j,k,l} \pm 0.2$	$2.6^{g,h} \pm 0.1$
	8	$4.7^{o,p} \pm 0.2$	$2.9^{h,i} \pm 0.1$
	16	$5.2^{p,q} \pm 0.1$	$3.6^{j,l,m} \pm 0.1$
	22	$5.7^{q,r} \pm 0.3$	$3.9^{m,n} \pm 0.1$
	30	$6.1^{r,s} \pm 0.1$	$4.2^{n} \pm 0.1$
WHT	**0**	$2.2^{b,c,d} \pm 0.1$	$1.0^{a} \pm 0.0$
	2	$2.5^{c,d} \pm 0.0$	$1.0^{a} \pm 0.0$
	4	$3.7^{f,g} \pm 0.2$	$1.2^{a,b} \pm 0.1$
	8	$4.8^{h,l,j,k} \pm 0.0$	$1.7^{c} \pm 0.1$
	16	$5.2^{j,k,l,m} \pm 0.0$	$2.4^{e,f,g} \pm 0.1$
	22	$5.8^{l,m,n} \pm 0.1$	$3.5^{l,j,l} \pm 0.2$
	30	$5.9^{m,n} \pm 0.1$	$3.6^{i,j,l} \pm 0.1$
TS	**0**	$1.9^{a,b,c} \pm 0.1$	$1.5^{a,b} \pm 0.3$
	2	$2.0^{a,b,c} \pm 0.1$	$1.7^{c} \pm 0.1$
	4	$2.2^{b,c,d} \pm 0.3$	$2.0^{c,d,e} \pm 0.1$
	8	$3.4^{e,f} \pm 0.4$	$2.6^{f,g} \pm 0.1$
	16	$3.8^{f,g} \pm 0.2$	$2.8^{g,h} \pm 0.2$
	22	$3.8^{f,g} \pm 0.1$	$3.2^{h,i} \pm 0.1$
	30	$4.2^{g,h} \pm 0.1$	$3.5^{i,j,l} \pm 0.1$
UV-C	**0**	$1.5^{a} \pm 0.0$	$1.0^{a} \pm 0.1$
	2	$1.7^{a,b} \pm 0.1$	$1.0^{a} \pm 0.1$
	4	$2.7^{d,e} \pm 0.1$	$1.2^{a,b} \pm 0.1$
	8	$3.6^{f,g} \pm 0.0$	$1.6^{b,c} \pm 0.1$
	16	$4.2^{g,h,l} \pm 0.1$	$2.2^{d,e,f} \pm 0.1$
	22	$4.7^{h,l,j} \pm 0.1$	$3.4^{i,j} \pm 0.1$
	30	$4.9^{i,j,k} \pm 0.1$	$3.5^{i,j,l} \pm 0.1$

Different letters in the same column indicated significant differences ($P < 0.05$)
Ctr (150 ppm at 5 ºC, pH 6.5, 2 min); US (45 kHz, 80%, 30 min); WHT (40 ºC, 30 min), TS (45 kHz, 80%, 40 ºC, 30 min) and UV-C (0.97 kJ.m^{-2})

Abstract

Previously optimized postharvest treatments were compared to conventional chlorinated water treatment in terms of their effects on the overall quality of tomato (cv. Zinac) during storage at 10 ºC. The treatments in question were; hot water treatment (WHT: 40 ºC, 30 min), ultrasounds (US: 45 kHz, 80%, 30 min), thermosonication (TS: 40 ºC, 30 min, 45 kHz, 80%) and ultraviolet irradiation (UV-C: 0.97 kJ.m-2). The quality factors evaluated were colour, texture, sensorial analysis, weight loss, antioxidant capacity, total phenolic content, peroxidase and pectin methylesterase enzymatic activities, and microbial load reduction.

The results demonstrate that all treatments tested preserve tomato quality to some extent during storage at 10 ºC. WHT, TS and UV-C proved to be more efficient on minimizing colour and texture changes with the additional advantage of microbial load reduction, leading to a shelf-life extension when compared to control trials. However, at the end of storage, with exception of WHT samples, the antioxidant activity and phenolic content of treated samples was lower than for control samples. Moreover, sensorial results were well correlated with instrumental colour experimental data.

This study presents alternative postharvest technologies that improve tomato (cv. Zinac) quality during shelf-life period, and minimize the negative impact of conventional chlorinated water on human safety, health and environment.

Keywords: Water heat treatment; ultrasounds; thermosonication; ultraviolet radiation; tomato; postharvest quality.