

Effect of ultrasound treatment on the quality and contents of polyphenols, lycopene and rutin in tomato fruits

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Abstract: The quality of tomato (*Solanum esculentum* L.) fruits is associated with their sensory characteristics and nutrient contents, among which are some secondary metabolites with biological activity. The aim of this study was to assess the effect of ultrasound (US) treatment on the quality and contents of polyphenols, lycopene and rutin in tomatoes after harvest. The application of US under controlled conditions of time and sonication amplitude (SA) induced a significant increase in polyphenol, lycopene and rutin contents in both the pulp and the skin of tomatoes at the beginning and end of the post-harvest evaluation period (0 and 6 days), standing out the 10 min 60% SA and 20 min 20% SA treatments. Additionally, with these treatments, no US effect was observed on the quality parameters (total soluble solids, titratable acidity, pH, firmness, and physiological weight loss). These results reveal that the US application to tomatoes is an attractive technology to increase the content of secondary metabolites in the fruit without affecting its quality.

Keywords: carotenoids; emerging technology; phytochemicals; *Solanum esculentum* L.

Phenolic compounds are phytochemicals widely distributed in the plant kingdom; they are secondary metabolites involved mainly in plant defences against ultraviolet radiation and pathogen aggression (Manach et al. 2004). Likewise, they are responsible to a great extent for the sensory characteristics of fruits and vegetables, such as colour, astringency, taste and aroma. In addition, these compounds present diverse biological functions, such as antioxidant, anti-inflammatory,

anticarcinogenic and vasodilator ones (Middleton et al. 2000). In turn, the large variety of colours found in fruits is due to the presence of carotenoids, which are phytochemicals that participate in the photosynthetic processes and provide protection against oxidative stress. Besides, carotenoids have shown beneficial effects in the prevention of chronic degenerative and cardiovascular diseases (Kadian & Garg 2012). Because the human body cannot synthesize these com-

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pounds, their presence is strictly associated with their intake through foods (Gómez-Romero et al. 2010).

The tomato (*Solanum esculentum* L.) fruit is considered one of the main agricultural products worldwide due to its nutritional potential and its high popularity level in all diets around the world. This fruit is an important source of polyphenols and carotenoids, among which stand out rutin and lycopene, respectively (Luna-Guevara et al. 2014). The rutin content in tomato is found at 14 to 20 mg 100 g⁻¹ dry basis and it has been recognized as an important compound for the human diet because it prevents aging due to cell damage by oxidative stress and for its anti-inflammatory activity (Valdez-Morales et al. 2014). In turn, lycopene is the most abundant carotenoid in tomato and is responsible for providing the characteristic red colour. The content between 37 and 46 mg 100 g⁻¹ dry basis has been reported (Ranveer et al. 2013); it has been associated with the prevention of prostate cancer and cardiovascular diseases (Yang et al. 2013).

In the last years, it has been demonstrated that ultrasound (US) can be a technology with a potential application in the food industry, mainly in the areas of processing and conservation (Ashokkumar 2015). Likewise, this technology considered as emerging has generated a great interest because it can be used in a practical way, with safe and environmentally sustainable equipment (Chemat et al. 2011). In this context, the US technology is a viable alternative for the preservation of fresh produce because of its promising effects in inactivating pathogenic microorganisms, as well as for the maintenance of quality parameters, nutritional components, and sensory characteristics. Until now, scarce information has been available on the use of US on fresh and processed tomatoes. Lianfu and Zelong (2008), using US-assisted extraction, obtained an increase in the yield of lycopene from a tomato paste, while Terefe et al. (2009) determined the inactivation of pectin methylesterase and polygalacturonase in tomato juice by thermosonication. Other studies demonstrated that US application in combination with commercial disinfectants (De São José et al. 2018) and with essential oregano oils (Luna-Guevara et al. 2015) induces antimicrobial and disinfecting features on cherry and Roma VF (*Verticillium* and *Fusarium* wilt resistant Roma variety) tomatoes, respectively. Based on the aforementioned, this study was aimed at investigating the effect of US application on the quality parameters and the content of polyphenols, lycopene, and rutin of tomato fruit after harvesting.

MATERIAL AND METHODS

Chemicals. Solvents (acetone, ethanol, methanol and hexane) and other reagents, including the phenolic standards (gallic acid and rutin hydrate), Folin & Ciocalteu's phenol reagent, lycopene standard and HPLC grade acetonitrile and methanol were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemical reagents used were of analytical grade.

Biological material. Healthy and physiologically mature Saladette tomato (*S. esculentum* L.) fruits were obtained from an orchard located in the municipality of Jala, State of Nayarit, Mexico. The fruits in the pink maturity stage [according to the classification proposed by Cantwell (2010); more than 30% but not more than 60% of the fruit surface in the aggregate shows pink or red colour] were washed with distilled water, dried and stored at room temperature until the fruits turned pink. The fruits were randomly sorted and divided into 21 groups (five fruits in each group; $n = 5$) for treatment with US.

US treatment. An ultrasonicator (UP400S; Hielscher Ultrasonics GmbH, Teltow, Germany) was used at a frequency of 24 kHz, power of 400 W and pulse cycle of 1 second. Different conditions of sonication amplitude (SA; 20, 40, 60 and 100%) and time (5, 10, 15, 20, 25 and 30 min) were evaluated at room temperature (Table 1). For the US treatment, the fruits of each group were completely submerged in a tub (capacity of 2.5 L) with distilled water; then the ultrasonicator was placed in the centre of the tub to apply US in the conditions of each treatment. Afterward, the best treatments were selected and the fruits were stored at 25 °C until further analysis.

Sample processing. The postharvest quality parameters were evaluated on day 0 and 6 of storage; during this period the tomato fruits reached the consumption maturity, turning fully red. Day zero was considered that day when the US treatment was applied and the determinations were performed 2 h after US application. For the analysis of total soluble solids, pH and titratable acidity, tomato fruits were cut into cubes of 2 cm³, homogenized with a mortar and pestle; subsequently, the juice obtained was filtered through a gauze filter. For the quantification of bioactive compounds, the skin and seeds of tomato fruits were manually separated from the pulp. Afterwards, the seeds were discarded and the samples were divided into pulp and skin fractions, frozen at -20 °C and lyophilized. Finally, the lyophilized samples were pulverized in a TissueLyser II mill (Quiagen, Hilden, Germany; $F = 30 \text{ L s}^{-1}$, during

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Table 1. Ultrasound conditions (time and sonication amplitude) evaluated in tomato fruit

Treatment No.	Ultrasound conditions	
	time (min)	sonication amplitude (%)
1	control	–
2	5	20
3	5	40
4	5	60
5	10	20
6	10	40
7	10	60
8	15	20
9	15	40
10	15	60
11	20	20
12	20	40
13	20	60
14	25	20
15	25	40
16	25	60
17	30	20
18	30	40
19	30	60
20	5	100
21	15	100

2.5 min), then they were stored in the dark at $-20\text{ }^{\circ}\text{C}$ until use in further analysis.

Determination of postharvest quality parameters

Total soluble solids ($^{\circ}\text{Brix}$). Total soluble solids were measured using a refractometer (HI 96811; Hanna Instruments, Woonsocket, RI, USA) at room temperature. The refractometer prism was washed with distilled water after each determination. Results were expressed in $^{\circ}\text{Brix}$.

pH determination. The pH was determined using a pH measuring glass electrode (HI2211-02, Hanna Instruments, Woonsocket, RI, USA) calibrated with commercial buffer solutions of pH 7.0 and 4.0. A 10 mL aliquot of the sample was placed into a 50 mL beaker with a magnetic stirrer and pH electrode inserted.

Titrateable acidity. Titrateable acidity was determined according to the AOAC (1984) method (Association of Official Analytical Chemists, USA). A 10 mL aliquot of the sample was placed into a 250 mL beaker and 90 mL of distilled water was added. The solution was continuously stirred by a magnetic stirrer and titrated against standardized 0.1 N NaOH using phenolphthalein as an indicator. The acidity of the

samples was expressed as percentage of citric acid according to the following equation:

$$\text{Acidity (\%)} = (\text{mL titrant base} \times \text{normality of base} \times \text{acid factor} / \text{mass of sample}) \times 100$$

Firmness. The firmness was measured in the equatorial region of each tomato fruit using a texture analyzer (TA-XT2i, Scientific Instrumentation, Quijorna, Madrid, Spain). The fruit was compressed to a depth of 5 mm, using a conical plate at a speed of 1 mm s^{-1} . Results were expressed in Newtons (N).

Weight loss. The physiological weight loss was determined by the difference between the weights of the fruits at the beginning and at the end (0 and 6 days), using a digital analytical balance. Results were expressed as percentage loss of the initial total weight.

Quantification of bioactive compounds

Polyphenol extraction. The extraction of polyphenols was performed according to the method by Saura-Calixto et al. (2007) with some modifications. Samples (0.5 g) of the tomato pulp or skin were mixed with 20 mL of 50% (v/v) methanol (containing 0.3 M HCl) and stirred for 1 h at room temperature. The mixture was centrifuged at 3 000 rpm for 10 min at $25\text{ }^{\circ}\text{C}$. The phases were separated and the supernatant was retained. The precipitate was washed with 20 mL of acetone/water (70 : 30 v/v) for 1 h and centrifuged under the same conditions, the supernatants from each extracted (methanol and acetone) were combined. Subsequently, solvents were removed in a rotary evaporator (RE100-Pro; DLAB Scientific, Riverside, CA, USA) at 60 rpm for 1 h at $45\text{ }^{\circ}\text{C}$. All extracts were stored at $4\text{ }^{\circ}\text{C}$ in dark conditions.

Total soluble polyphenol content. The total soluble polyphenol (TSP) content was determined spectrophotometrically by the Folin-Ciocalteu method according to Singleton et al. (1999), using gallic acid as standard ($0\text{--}300\text{ mg L}^{-1}$). Results were expressed as milligram equivalents of gallic acid per gram of extract (mg GAE g^{-1}). Thereafter, the concentration of rutin and lycopene metabolites was determined in those treatments that induced the highest TSP content.

Lycopene determination. Lycopene determination was performed following the procedure described by Sadler et al. (1990) with some modifications. In a 2-mL Eppendorf tube, 0.1 g of lyophilized sample of tomato pulp or skin was supplemented with 500 μL of ethanol (containing BHT at 0.1% w/v) and heated at $80\text{ }^{\circ}\text{C}$ for 5 min. Then, 10 μL of 80% KOH (w/v) was

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added and agitated for 1 min and heated at 80 °C for 10 min. The mixture was cooled on ice for 5 min and supplemented with 250 µL of hexane and 500 µL of deionized cold water (tube 1), the mixture was shaken and centrifuged at 3 500 rpm during 10 min at 4 °C. The recovered supernatant was placed in a new tube (tube 2), avoiding its exposure to light. Afterward, two more washes were performed under the same conditions, collecting the supernatants in tube 2. From the supernatant solution, a 400-µL aliquot was taken and filtered (using a 0.2 µm filter, diameter 13 mm; Whatman No.7402-001; Whatman, Maidstone, UK). Lycopene was determined in a HPLC (Dionex UltiMate 3 000; Thermo Scientific, Sunnyvale, California, USA) system equipped with a titanium quaternary pump (LPG-3400AB), an autosampler (WSSIN-3 000TBPL), a photodiode array detector (DAD-3 000; Thermo Fisher Sci., New York, NY, USA) and Chromeleon 7.0 Software (Dionex; Thermo Fisher Sci., New York, NY, USA); using an Acclaim Polar Advantage II, C18 reverse phase column (150 mm × 2.1 mm i.d., 3 µm; Thermo Fisher Sci.; New York, NY, USA). The mobile phase (isocratic) was: acetonitrile-methanol-methylene chloride (40 : 55 : 5 v/v/v), containing 0.05% trimethylamine and 0.1% BHT. The flow rate was 0.5 mL min⁻¹, absorbance was registered at 473 nm. Lycopene was identified by comparing the retention time and absorption spectrum with a pure (≥ 85%) analytical standard of lycopene (Sigma-Aldrich; St. Louis MO, USA); a calibration curve of the standard was constructed (5–50 µg mL⁻¹). Each sample was analyzed in triplicate (reinjecting once) in two independent experiments (CV ≤ 30%).

Rutin determination. Rutin determination was performed following the procedure described by Capanoglu et al. (2012) with some modifications. Starting with 0.5 g of a lyophilized sample of tomato pulp or skin, the first wash was performed adding 10 mL of 80% ethanol, then the sample was centrifuged at 4 000 rpm during 10 min at 4 °C, the supernatant was recovered, and 10 mL of the ethanol solution was added to the initial tube to continue with the next four washes under the same conditions. From the supernatant solution, a 400-µL aliquot was taken, filtered (using a 0.2 µm filter, diameter 13 mm), and analyzed in the HPLC system (Dionex UltiMate 3 000) described above. The separation was carried out with an Acclaim 120 A C18 column (250 mm × 4.6 mm i.d., 5 µm; Thermo Fisher Sci.; New York, NY, USA). The separation was performed according to Espinosa-Alonso et al. (2006) with some modifications, injecting 10 µL of sample, and the mobile phase consisted

of two solvent systems: A = water acidified with acetic acid (pH 2.8) and B = acetonitrile at room temperature. The gradient of elution was: 95% A and 10% of B from 0 to 2.5 min, from 10 to 12% of B from 2.5 to 6 min, from 12 to 23% of B from 6 to 18 min, from 23 to 35% of B from 18 to 24 min, from 35 to 95% from 24 to 30 min, and returning to the initial conditions at 40 min. The flow rate was 0.5 mL min⁻¹ and the fixed wavelength was 360 nm. Chromatographic peak was identified by comparing the retention time and UV-visible absorption with a rutin hydrate (pure ≥ 95.0%) analytical standard (Sigma-Aldrich; St. Louis, MO, USA); a calibration curve of the standard was constructed (1–30 µg mL⁻¹). Each sample was analysed in triplicate (reinjecting once) in two independent experiments (CV ≤ 30%).

Statistical analysis

Results are reported as means ± standard deviation. Significant differences between treatments were determined by analysis of variance and Fisher's least significant difference (LSD) test using the Statistica 10 (StatSoft; Palo Alto, CA, USA). Statistically significant differences were considered at $P < 0.05$.

RESULTS AND DISCUSSION

Fruit quality. We evaluated different time and SA conditions to select the US treatment that would not affect the physical integrity of the fruit, and to discard those treatments that caused evident physical damage. Treatments 4, 7, 11, and 14 (Table 1) were the chosen ones which correspond to 5 min 60% SA, 10 min 60% SA, 20 min 20% SA, and 25 min 20% SA, respectively. In fruits treated under these conditions, quality parameters (°Brix, pH, titratable acidity, firmness and weight loss) were determined. Results are given in Table 2, which reveals that there were no significant differences ($P < 0.05$) between treatments. Hence, under the selected conditions, the implosion of bubbles (acoustic cavitation) formed by the US treatment did not induce any evident physical damage to the tomato fruits. Likewise, a normal development of the ripening process of the fruit was observed, because °Brix, pH, titratable acidity, firmness, and weight loss values on day 6 did not show a significant difference ($P < 0.05$) from the control and remained within the range previously reported for tomatoes (Hernández-Leal et al. 2013; Valdez-Morales et al. 2014; Ponce-Valadez et al. 2016). Based on the aforementioned, it can be inferred that under the selected conditions, the cavitation process

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Table 2. Quality parameters of tomato fruit treated with ultrasound on different days of storage (mean \pm SD; $n = 5$)

Parameter/Days	Ultrasound conditions											
	control		5 min 60%		10 min 60%		20 min 20%		25 min 20%			
	0	6	0	6	0	6	0	6	0	6	0	6
Acidity (%)	1.11 \pm 0.03 ^a	0.55 \pm 0.06 ^b	1.15 \pm 0.04 ^a	0.48 \pm 0.05 ^b	1.14 \pm 0.03 ^a	0.49 \pm 0.08 ^b	1.11 \pm 0.06 ^a	0.52 \pm 0.05 ^b	1.12 \pm 0.03 ^a	0.56 \pm 0.03 ^b		
^a Brix	6.83 \pm 0.07 ^a	8.52 \pm 0.18 ^b	6.73 \pm 0.17 ^a	8.41 \pm 0.62 ^b	6.74 \pm 0.21 ^a	8.79 \pm 0.05 ^b	6.91 \pm 0.03 ^a	8.53 \pm 0.14 ^b	6.72 \pm 0.24 ^a	8.89 \pm 0.05 ^b		
pH	4.36 \pm 0.04 ^a	4.44 \pm 0.03 ^b	4.34 \pm 0.32 ^a	4.46 \pm 0.06 ^b	4.32 \pm 0.01 ^a	4.42 \pm 0.01 ^b	4.33 \pm 0.05 ^a	4.43 \pm 0.02 ^b	4.28 \pm 0.04 ^a	4.45 \pm 0.05 ^b		
Firmness (N)	31.09 \pm 0.26 ^a	16.59 \pm 0.31 ^b	31.41 \pm 0.32 ^a	17.71 \pm 0.15 ^b	31.09 \pm 0.81 ^a	16.29 \pm 0.76 ^b	30.89 \pm 0.65 ^a	17.39 \pm 0.89 ^b	31.49 \pm 0.41 ^a	16.32 \pm 0.49 ^b		
Weight loss (%)	0.89 \pm 0.18 ^a	4.15 \pm 0.21 ^b	0.81 \pm 0.14 ^a	4.21 \pm 0.06 ^b	0.79 \pm 0.14 ^a	4.29 \pm 0.45 ^b	0.79 \pm 0.17 ^a	4.25 \pm 0.65 ^b	0.79 \pm 0.16 ^a	4.37 \pm 0.03 ^b		

Ultrasound conditions show the time of application and the sonication amplitude; SD – standard deviation; values that have the same superscript in a parameter do not differ significantly ($P < 0.05$)

is not determinant to increase the permeability of the membrane and, consequently, it does not increase the respiration velocity of the fruit. Previous studies reported that US treatment can inhibit the respiration rate of cherry tomatoes (Wang et al. 2015), pears (Zhao et al. 2007) and plums (Chen & Zhu 2011) in the post-harvest stage, inducing a delay in the ripening of the fruit. In turn, it has been observed that US treatment can delay a diminution in the firmness of peaches (Wang et al. 2006), strawberries (Cao et al. 2010) and asparagus (Wei et al. 2013). Although our results do not suggest a delay in the ripening of the fruit, the quality attributes did not differ significantly ($P < 0.05$) from the control, therefore we continued to evaluate the US effect on the content of secondary metabolites in tomato fruits under the selected conditions.

TSP content. The TSP content in the pulp showed no significant difference ($P < 0.05$) from the control or between treatments (data not shown). However, in the skin a significant increase ($P < 0.05$) was observed in comparison with the control on day 6 with treatments of 10 min 60% SA and 20 min 20% SA, when a TSP increase of 165% and 243%, respectively, was observed (Figure 1). The aforementioned suggests a response of the fruit to US treatment, inducing the synthesis of phenolic compounds, which are secondary metabolites that function as defence mechanisms. In this sense it is known that plant systems, because of being sessile organisms, respond rapidly and coordinately to any stimulus of either biotic or abiotic nature (Manach et al. 2004). The plant organisms have evolved a number of strategies to respond to an abiotic stimulus such as UV-B radiation, these include a variety of soluble flavonoid pigments that are typically localized within the vacuoles of epidermal cells, phenolic compounds present in the polysaccharide cell wall, and lipophilic phenolic molecules that are covalently bound to cutin (Pfundel et al. 2006). Results show that the most effective treatments were 10 min 60% SA and 20 min 20% SA, indicating an inverse relation between time and SA, i.e. with a shorter time it is more effective to apply a higher SA, and with a longer time it is convenient to use a lower SA to generate an inductive stimulus without affecting the integrity of the fruit. Valdez-Morales et al. (2014) reported a TSP value of 19.3 mg GAE g^{-1} in the ripe tomato skin, when significantly higher concentrations were obtained in this study due to the application of US. Based on the aforementioned, we decided to assess the US effect on the content of specific secondary metabolites of tomato, like lycopene and rutin, under the treatments that were the most ef-

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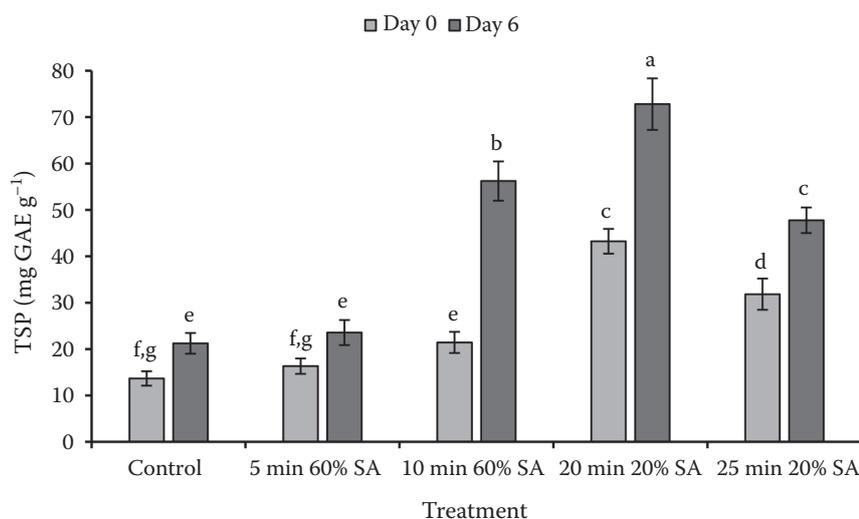


Figure 1. Ultrasound effects on the content of total soluble polyphenols (TSP) in the tomato skin on days 0 and 6 post-treatment (mean \pm SD; $n = 5$)

Different letters in the columns represent statistically significant differences ($P < 0.05$); GAE – equivalents of gallic acid; SA – sonication amplitude

fective on the TSP content: 10 min 60% SA and 20 min 20% SA.

Lycopene content. Figure 2 reveals a significant difference ($P < 0.05$) between the assessed treatments and the control. On day 0, both in pulp and skin a lycopene increase by approximately 100% and 200% was observed under 10 min 60% SA and 20 min 20% SA treatments, respectively, in comparison with the control. On day 6, in the pulp, a 42% and 97% increase was observed under 10 min 60% SA and 20 min 20% SA treatments, respectively. Regarding the skin on day 6, an increase by 72% and 118% was observed under treatment conditions of 10 min 60% SA and 20 min 20% SA, respectively. The lycopene concentration is very variable among tomato

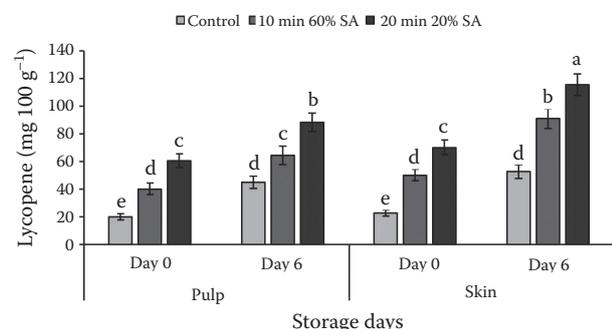


Figure 2. Ultrasound effects on the content of lycopene in tomato pulp and skin on days 0 and 6 post-treatment (mean \pm SD; $n = 5$)

Different letters in the same parameter (pulp or skin) indicate significant differences ($P < 0.05$); SA – sonication amplitude

cultivars, because different factors influence them, such as growth conditions, type of cultivar, and maturity stage of the fruit. In the present study, lycopene content in tomato (control) was within the previously reported range (37–46 mg 100 g⁻¹) in red varieties (Ranveer et al. 2013), this validates our results and also shows that lyophilization is a convenient method to prepare the sample for lycopene determination. Ryckeboosch et al. (2011) reported that lyophilization does not affect the carotenoid stability. On day 0 the lycopene concentration was very similar in tomato pulp and skin, although it increased in a proportional manner when the US treatment was applied. According to results, it can be argued that the fruit responds rapidly to the stimulus, inducing biosynthesis of secondary metabolites, among them of lycopene, which contributes to the defence of the fruit. On 6 day the lycopene concentration was significantly higher in the skin compared with the pulp, which suggested that the first US stimulus was sufficient to keep the fruit on alert, increased the metabolite concentration in the skin involved in defence because the skin together with the cuticle is the first line of defence against biotic and abiotic stimuli (Yeats & Rose 2013). It has been reported that another abiotic stimulus like UV radiation increased the content of secondary metabolites for plant protection (Pfündel et al. 2006). It was possible to reach a significant increase in the lycopene content due to US application.

Rutin content. In tomato, the main flavonoid is rutin with an average content of 20 mg 100 g⁻¹, which agrees with the value obtained in the control sample of this

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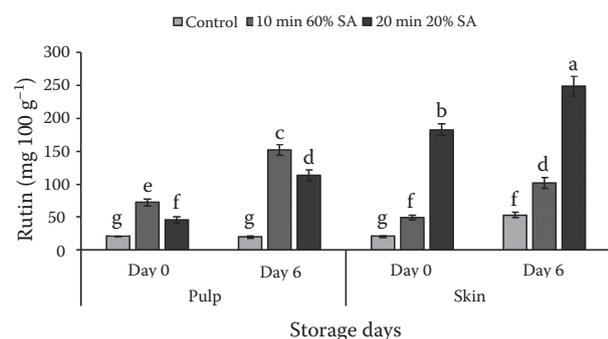


Figure 3. Ultrasound effects on the content of rutin in tomato pulp and skin on days 0 and 6 post-treatment (mean \pm SD; $n = 5$)

Different letters in the same parameter (pulp or skin) indicate significant differences ($P < 0.05$); SA – sonication amplitude

study (Figure 3). A significant difference ($P < 0.05$) was observed between treatments, when on day 6, after the 10 min 60% SA treatment, the sevenfold rutin content was obtained in the pulp compared to the control. On the other hand, in the skin, on day 6, the 20 min 20% SA treatment resulted in a 360% rutin increase as compared to the control. Metabolically, rutin is located in the last stages of the flavonoid biosynthesis pathway and is related to the consumption maturity stage of the fruit (Valdez-Morales et al. 2014). Due to US application, a significant increase of this metabolite was achieved, obtaining a higher effect on the skin of the fruit, which is the first protection barrier; in this way we could infer that rutin induction in the skin potentiates the protecting effect in the fruit against the US-induced stress. So the US treatment could be a way to increase the nutritional value of tomato fruits.

CONCLUSION

The US application increased the contents of TSP, lycopene, and rutin significantly. Additionally, the US application did not affect the post-harvest quality of tomato fruits. These results suggest that the US application to the tomato fruit is an attractive technology to stimulate the biosynthesis of TSP, lycopene, and rutin, contributing to an improvement in the nutritional value without diminishing the quality of the fruit. On the other hand, more detailed studies must be performed focused on the analysis of the expression of genes that code for the enzymes involved in the biosynthesis of secondary metabolites, as well as to evaluate the activity of key enzymes that participate in the metabolic pathways, aiming at elucidating the possible mecha-

nisms of metabolite induction by the application of US as an abiotic agent.

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