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Phytonutraceutical evaluation of five varieties of tomato (*Solanum lycopersicum*) during ripening and processing

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ABSTRACT

Tomato (Solanum lycopersicum) is the largest produced vegetable in the world after potato and sweet potato. It is an important source of carotenoids (mainly lycopene), ascorbic acid, and phenolic compounds. In this work, we evaluated five tomato varieties grown in Costa Rica, during ripening, by their capacity to produce the compounds mentioned above and their antioxidant and antimicrobial activity. Additionally, we evaluated the decay of the content of metabolites during the agro-industrial processing and the revalorizing of agricultural byproducts from the tomato industry, as sources of antioxidant compounds. The JR variety shows the highest lycopene concentration, $243 \pm 7 \, \mu g/g$, while the highest concentration of this metabolite in the paste corresponded to variety 1710 with a value of $238 \pm 7 \, \mu g/g$. Variety 115 showed the highest concentration of carotenoids in fresh fruit, post-harvest fruit, and tomato paste $(4.1 \pm 0.6, 2.42 \pm 0.08, \text{ and } 1.84 \pm 0.01 \, \text{mg/g}$, respectively). The highest content of total phenols was obtained in leaves of the 115 variety, with a concentration of $9.0 \pm 0.2 \, \text{mg GAE/gDS}$. We also demonstrated that leaves are a valuable source of phenolic antioxidants. Additionally, there is a demonstrated antimicrobial capacity in some ethanolic extracts of tomato, and the spectrum of action depends on the variety and the ripening of cherry-type varieties.

1. Introduction

Tomatoes are the vegetables with the largest volume of production in the world after potato and sweet potato (180 million MT in 2019) (FAO, 2019). They are widely consumed in many diets and countries worldwide and constitute an important source of nutrients and phytonutraceuticals. Tomatoes are especially rich in carotenoids (mainly lycopene), vitamin C, and polyphenolic compounds (Farooq et al., 2020). These compounds have been linked to healing or preventive performance against diseases such as chronic cardiovascular, neurodegenerative, inflammatory, atherosclerotic pathologies and some types of cancer (Tamasi et al., 2019).

Lycopene is the main carotenoid in tomatoes, usually comprising approximately 80–90% of them (Shi, Maguer, & nutrition, 2000). Carotenoids are antioxidants due to their polyunsaturated conjugated

system, which can interact with Reactive Oxygen Species (ROS), such as hydroxyl radical (\cdot OH), superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen (1O_2) (Tatsuzawa, Maruyama, Misawa, Fujimori, & Nakano, 2000). However, lycopene sets apart the highest singlet oxygen quenching rate among all dietary antioxidants we can find in human plasma (Tatsuzawa et al., 2000). Lycopene has shown therapeutic-oxidative stress reduction, neuronal apoptosis and inflammation reduction, and restoration of mitochondrial functions. It helps prevent and treat Parkinson's, Huntington's, Alzheimer's, epilepsy, depression, cardiovascular diseases, heart failure, neoplasms, and lung and prostate cancers (Saini, Rengasamy, Mahomoodally, & Keum, 2020).

The characteristic red color of tomatoes develops due to the accumulation of lycopene (red pigment) and the degradation of chlorophylls, which turn from green to white. Biosynthesis of lycopene is upregulated

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 Table 1

 Origin and characteristics of tomatoes utilized in this study.

Name	Other names (full name)	Country of origin	Type and characteristics	References
115	INTA-	Panama	Cherry type, red-	(L. Lopez-Marín,
	115		colored, it can be	2017)
117	INTA-	Panama	freely re-cultured and	
	117		crossed by the	
			producers, it is not	
			commercially sold.	
1710	FBM-	Costa	Autochthonous hybrid	(Lopez-Marín,
	17-10	Rica	round-shaped fruit,	Brenes-Peralta,
1713	FBM-	Costa	developed by the	Jiménez-Morales, &
	17-13	Rica	"Fabio Baudrit	Gonzalez-Masis, 2019)
			Moreno Experimental	
			Agronomic Station"	
			(EEAFBM by its	
			Spanish Acronym).	
			Red-colored, bigger	
			than 260 g.	
			R. solanacearum &	
			F. oxysporum resistant	
			fruits. The average	
			production is 8 kg per	
			plant. It is TYLCV susceptible.	
JR	JR	USA	Undetermined, extra	(OFINASE, 2021)
) IC		USA	firm. From Gargiula,	(OFINASE, 2021)
	special		Inc./BHN Seed	
			mc./ Drin Seeu	

during ripening, and enzymes converting lycopene into other metabolites are dramatically downregulated (Ronen, Cohen, Zamir, & Hirschberg, 1999). Lycopene and β -carotene belong to the same poly-cis biosynthetic pathway: geranylgeranyl diphosphate (GGPP) \rightarrow 15-cis-phytoene \rightarrow 9,15,9'-tri-cis-ζ-carotene \rightarrow 9,9'-di-cis-ζ-carotene \rightarrow prolycopene \rightarrow all-trans-lycopene \rightarrow β -carotene; catalyzed by phytoene synthase (PSY), phytoene desaturase (PDS), ζ -carotene isomerase (ZISO), ζ -carotene desaturase (ZDS), carotene isomerase (CrtISO), and lycopene β -cyclase (LCYB), respectively (Zhang, Li, Tu, Cheng, & Yang, 2018). Then, a small amount of lycopene is converted into other carotenoids, such as α -carotene, and β -carotene.

Over 20 carotenoids have been characterized in tomatoes, such as α -, β -, γ - and ξ -carotene, phytoene, phytofluene, neurosporene, and lutein (Shi, Kakuda, & Yeung, 2004). Carotenoids are involved in synergistic therapeutic effects, and some are provitamins as well (Abushita, Daood, & Biacs, 2000). Other antioxidants such as polyphenols are present in tomatoes at lower concentrations, such as hydroxycinnamic acids, flavanones, flavonols, and anthocyanins (Martí, Roselló, & Cebolla-Cornejo, 2016). Ascorbic acid, or vitamin C, is present in tomatoes in moderate amounts compared to other fruits, although they are an important source of this vitamin because of their high consumption rate (Stevens, Buret, Garchery, Carretero, & Causse, 2006).

The concentration of carotenoids, vitamin C and polyphenolic compounds are not the same for different tomato varieties. In Costa Rica, several tomato genotypes have been identified with significant variability of metabolites (Monge-Pérez, 2014). Seed phenotypical development is mainly studied in developed countries, but imported seeds do not show the same performance in tropical countries. Also, tomato post-harvest plant tissues such as leaves and stems are known to contain a significant amount of some of the metabolites present in the fruits as well; however, these materials are basically wasted. This work seeks to evaluate the nutraceutical and antimicrobial potential of five varieties of tomato, including imported, freely-reproduced, and autochthonous developed hybrid seeds at different ripening and post-harvest conditions, and as processed paste; moreover, agricultural wastes such as leaves and stems were included. Our further purpose is the obtainment of better functional tomatoes and processed products.

2. Materials and methods

2.1. Materials

Five tomato varieties (115, 117, 1710, 1713, and JR) have been studied; these were used commercially or in on-site testing at the Costa Rican tomato subsector farms. Materials coded as 115 & 117 (both cherry types) were collected in Tobosí, Cartago (1400 mamsl (meters above the medium sea level)). 1710, 1713, and JR (all of them rounded type) were sampled at San Pedro and Santa Bárbara, Heredia Province (1100 mamsl). Characteristics of tomatoes are detailed in Table 1. Each variety was collected at three ripening stages (G1-green, G3-turning, and G6-red) according to the standards of the United States Department of Agriculture (USDA, 1991). Post-harvested on shelves (PH), and paste (Sa) were included. Pasta processing began with selection, chunking, and scalding in boiling water (for color fixation and enzyme deactivation). Then, the material was de-pulped and cooked until it reached 17°Brix. Finally, citric acid, salt, and preservatives were added. In addition, post-harvest stems (St) and leaves (L) were collected. All samples were cut into pieces, dehydrated in a freeze-dryer Freezone 2.5 Plus (from Labconco Corp., Kansas City, MO), and powdered. Plant tissues (L & St) were used for polyphenol analysis and antioxidant activity, and fruit samples (G1, G3, G6, PH & SA) were analyzed for lycopene, vitamin C, and total carotenoids, as well as those mentioned for tissues. Samples were stored at 5 °C in the dark.

2.2. Ascorbic acid determination

Ascorbic acid (vitamin C) was determined by an iodometric method, as a modification of the method described by Sapei and Hwa (2014). 300 mg of tomato samples described hereinabove were extracted with 3 mL of distilled water into an ultrasonic bath for 10 min. Then, the extract was centrifuged for 2 min at 840×g in a centrifuge model ST8R (from Thermo Scientific, Waltham, MA). The solid was discarded, and the supernatant was transferred into a 100 mL Erlenmeyer flask. 30 mL of water and 2 mL of 1% starch indicator were added. Samples were titrated with 0.01 N $\rm I_2/KI$ solutions which were previously standardized with sodium thiosulfate. Three replicates were performed.

2.3. Total phenolic content determination

0.1000 g of each tomato fruit and paste and 0.0500 g of each leaf and stem were individually mixed with 2 mL 95% ethanol and placed into an ultrasonic bath for 10 min. Then, the mixture was centrifuged at $840\times g$ for 2 min. The supernatant was acidified by adding 2 drops of 0.1 mol/L HCl solution and the volume was adjusted to 10 mL. At least four replicates were prepared. Finally, total phenolic content was analyzed using Folin-Ciocalteu's colorimetric method, as described in our previous report (Syedd-León, Orozco, Álvarez, Carvajal, & Rodríguez, 2020). Every 30 μL of each previously prepared sample was mixed with 200 μL of water into a 96-well microplate. Then, 15 μL of Folin-Ciocalteu reagent, and 50 μL of 20% carbonate sodium solution were added to the microplate. The plate was incubated for 20 min with agitation in a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments) at 40 °C. After incubation, absorbance was measured at 755 nm, against 0.000, 0.020, 0.040, 0.060, 0.080, 0.120 mg of gallic acid/1 mL.

2.4. Lycopene quantification

0.1 g of each sample was extracted with 2 mL 50:50 tetrahydrofuran (THF)/hexane, vortexed, and placed into an ultrasonic bath for 3 min. Then, the mixture was centrifuged for 15 min at (840×g). The procedure was repeated 3 times in order to collect approximately 9 mL. Then, the three extracts together were dried into a *SpeedVac Concentrator* SPD1010 (from Thermo Fisher Scientific, MA, USA). The resulting dry powder was dissolved into 500 μ L of THF and analyzed into a liquid

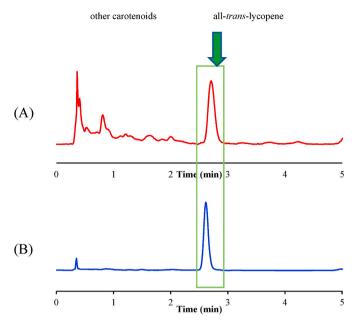


Fig. 1. Chromatographic separation of lycopene by HPLC with diode array detector at 474 nm of: (A) Extract of tomato 1713 in hexane: THF (50:50), (B) Lycopene standard.

chromatographer model Prominance (from Shimadzu Corp., Kyoto, Japan), equipped with a C30 Acclaim column (150 \times 3.0 mm y 5 μm ; Thermo Fisher Scientific) and diode array detector. Analysis was performed at a wavelength of 474 nm, a run time of 5 min, and room temperature. The mobile phase was ethanol, methanol, and THF (15:5:1) pumped at a flow rate of 1.5 mL/min (isocratic). Lycopene concentration was determined against a 0,0000, 0.0008, 0.0024, 0.0064, 0.0160, 0.0400 mg/mL calibration curve. Samples were analyzed by duplicates. Fig. 1 shows the chromatogram of sample 1713 and the standard (see Fig. 2).

2.5. Total carotenoid quantification

0.1 g of samples were extracted with 2 mL of a mixture of hexane and ethyl acetate (50:50) into an ultrasonic bath for 15 min. The same solvent was utilized during all the analyses. The procedure was repeated 3 times, and the supernatants were mixed and adjusted to 10 mL. Solutions were stored in dark containers at 5 °C. Total carotenoids were determined by colorimetry at 449 nm in a T80 + UV/Vis spectrophotometer (from PG Instruments Ltd., Lutterworth, UK) against a calibration curve in the range of 0–45,00 µg/mL β -carotene. The pure β -carotene standard was obtained by extracting 2 kg of freeze-dried carrots in hexane at an ultrasonic bath for 20 min, then concentrating, purifying in a silica gel flash column eluted with hexane, and drying in a rotatory evaporator, as previously reported (Shibata, Ishihara, & Matsumoto, 2004). Quantification was performed in a duplicate.

2.6. Antioxidant activity determination

The antiradical activity against DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical was determined following our previously published protocol with some modifications (Araya, Carvajal, Alvarez, Orozco, & Rodriguez, 2017). Trolox is used as standard for DPPH free radical scavenging assay, and results are expressed as Trolox equivalent (TE). 0.1000 g of tomato samples were macerated with ethanol 95% for 10 min into an ultrasonic bath. Then, the mixture was centrifuged for 2 min (840×g). After centrifugation, extracts were stabilized with 2 drops of HCl 0.1 mol/L. The procedure was repeated five times, then, extracts were mixed, and the volume was adjusted to 10 mL. Later, 0.0500 g of

samples of leaves and stems were extracted, using the same procedure but extracting four times instead of five. 30 μL of each extract were individually mixed in microplate wells with 270 μL of 0.042 mg/mL DPPH- solution, previously prepared using 80%v/v methanol as solvent. Solutions were incubated for 20 min and TE was determined by recording the absorbance at 515 nm against a 0–350 μM Trolox Standard Curve, in a microplate reader BiotekSynergy HT.

2.7. Determination of antimicrobial activity

Tomato fruits samples were evaluated in terms of their antimicrobial activity against four common strains: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 9027. 9 g of tomato samples were extracted with 100 mL of ethanol 95%, into an ultrasonic bath for 10 min. Then, the solution was filtered, concentrated in a rotary evaporator, and freeze-dried. Finally, the relative percent of inhibition was determined following the Kirby-Bauer's test, as previously described (Syedd-León et al., 2020).

2.8. Statistical analysis

Either numerical or graphical results were calculated using mean values and standard deviations (M \pm SD). Multiple comparisons using ANOVA and Post-hoc Tukey's tests were performed for the analysis of the concentration of lycopene, carotenoids, vitamin C, and total phenolics, as well as antioxidant, and antibiotic activity. The graphic analysis of all pairwise comparisons with the Tukey method was performed using RStudio as an integrated development environment for the R programming language (version 4.0.4) (R Core Team, 2021). The Tukey confidence limits for all pairwise comparisons were estimated with 95% of confidence.

3. Results and discussion

3.1. Carotenoid content accumulation

Fig. 2 (A–H) & (F-J) describes lycopene and total carotenoids as β -carotene contents (respectively) for the five varieties of tomato. All of them showed a significant increase in both lycopene and total carotene concentrations as ripening advances from G1 to G6; although concentrations of lycopene in varieties 117, 1710, and JR, and concentrations of β -carotene in variety 115 were not significantly different during the two first stages (G1 and G3). After harvesting or saucing, β -carotene's concentration decreased in varieties 115, 117, and JR; and remained constant in 1710 and 1713.

According to literature, lycopene concentration is influenced by whether or not the state of optimal maturation is yielded at harvest (Bui, Makhlouf, & Ratti, 2010): lycopene is reported to increase exponentially, in a higher order of magnitude when the fruit is harvested before complete maturity (Baldwin, Scott, Shewmaker, & Schuch, 2000). This behavior was similar to the one observed in our results for varieties 1710 and JR. However, a decreasing lycopene content trend after harvesting was observed in variety 115. This is explained due to, degradation processes that start taking place after the optimal ripening stage, being more severe in some varieties (Bui et al., 2010). Varieties 117 and 1713 had similar lycopene at PH, with a nominal but not significant decrease.

Processing of tomatoes, such as grinding, canning, cooking, or others, can promote accelerated oxidation and/or *cis*-isomerization of the lycopene but also can increase bioavailability by disrupting cell membranes (Martínez-Hernández et al., 2016). In our samples, all the varieties of tomato that had already started decreasing the concentration of lycopene after harvest, continuing with the same trend during paste processing. Pasta from 115, 117, and 1713 had a lower lycopene content than G6. This was explained because, during the scalding process, the mixture was heated at 100 °C for 3–5 min for color fixation, partially

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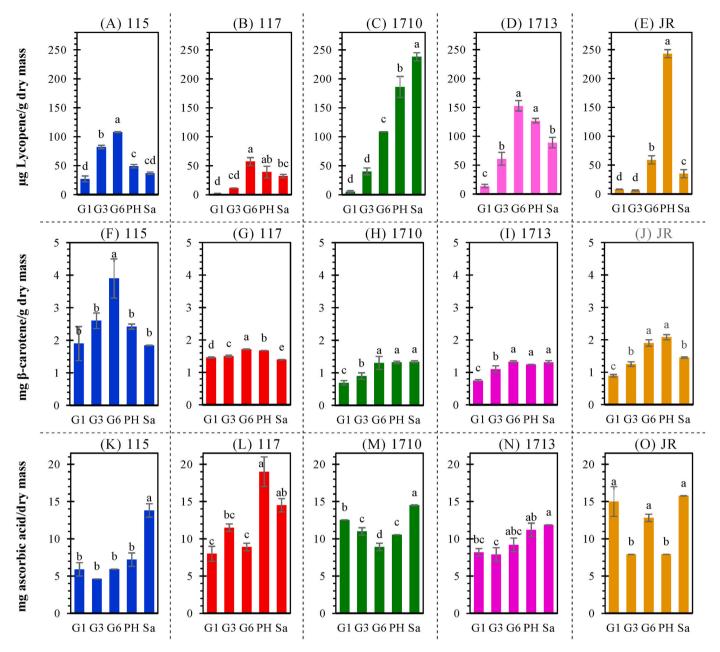


Fig. 2. Lycopene (A–E), total carotenoids (F–J) and vitamin C (K–O) concentrations in samples of five varieties of tomatoes: 115 (A, F, & K), 117 (B, G, & L), 1710 (C, H, & M), 1713 (D, I, & N) and JR (E, J, & O), at three maturation conditions (G1, G3, & G6), post-harvest (PH) and paste (Sa). Error bars represent standard deviation. G1, G2, and G3 represent different maturity conditions; PH and Sa stand for "post-harvest" and paste, respectively. Letters on top of columns represent compact letter displays of Tukey's Honest Significant Difference.

degrading the lycopene. In a previous study, isomerization of all-trans-lycopene into cis-lycopene was reported after 12 min (Manzo, Santini, Pizzolongo, Aiello, & Romano, 2019), but this was also depending on the characteristics of the variety and the process itself. The JR variety yielded the maximum value at the post-harvest condition, but it decreased significantly when it was converted into paste. The 1710 variety continued increasing lycopene concentrations on paste, which probably means enzymes responsible for ripening are still active during the processing of the material, and oxidation was not as severe on those specimens.

Lycopene had a maximum concentration of approximately 240 μ g/g in JR and 1710 varieties. When we compared the maximum lycopene concentrations of the varieties, regardless of ripening stage, those varieties reached a value 1.6 times higher than variety 1713; 2.2 times higher than 115, and 4.2 times higher than 117.

3.2. Vitamin C during ripening and processing

Fig. 2 (K–O) describes the change in citric acid concentration over the ripening and processing stages. We did not find a significant difference in ascorbic acid concentration during the three stages of ripening of tomatoes 115, 117, and 1713. There was not a clear trend in the JR variety. Ascorbic acid in the variety 1710 significantly decreased from stages G1 to G3. According to literature, the decline in vitamin C in tomatoes happens just after the ripening starts, because of its conversion into dehydroascorbic acid by the ascorbate oxidase enzyme (Martínez-Hernández et al., 2016).

Tomato processing usually involves a severe degradation of ascorbic acid (Demiray, Tulek, & Yilmaz, 2013) due to several factors, such as pH, moisture content, oxygen, temperature, and metal ion catalysis (Uddin, Hawlader, & Zhou, 2001). Interestingly, tomato 117 showed

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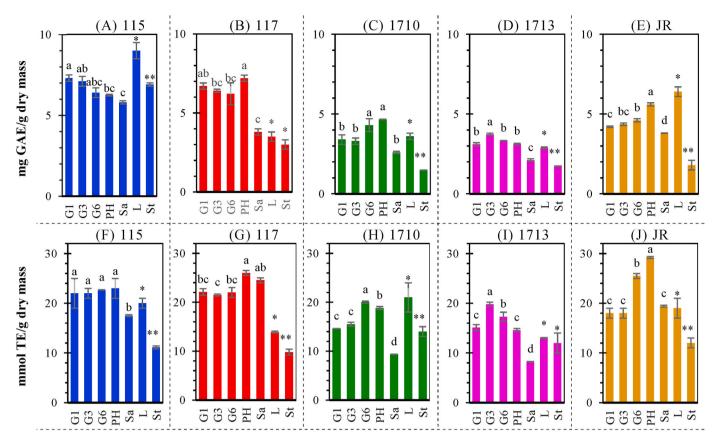


Fig. 3. Total phenolic compounds (A–E), and 2,2-Diphenyl1-picrylhydrazyl radical (DPPH) assay (F–J) expressed as Gallic Acid Equivalents (GAE), and Trolox Equivalent Antioxidant Capacity (TE), respectively, in samples of five varieties of tomatoes: 115 (A & F), 117 (B & G), 1710 (C & H), 1713 (D & I) and JR (E & J). Samples include fruits and tissues. Fruits at three maturation conditions (G1, G3, & G6), post-harvest (PH), and paste (Sa) were included, as well as tissues of leaves (L) and steams (St). Error bars represent standard deviation. Letters and asterisks on top of columns represent two independent compact letter displays of Tukey's Honest Significant Difference, for fruits and tissues, respectively.

greater ascorbic acid concentration in post-harvested than in pre-harvest conditions. Varieties 115 and 1713 had a similar amount of citric acid at post-harvest, and 1710 was slightly higher than G6. Enzymatic machinery in variety 1710 seemed to be less affected by degradative or oxidative processes than the other tomatoes evaluated when it was harvested under the conditions of this study. Actually, 1710 kept increasing lycopene and ascorbic acid on the shelves and in the processed product and showed a plateau at the highest total carotenoid concentration. Following these results, we can hypothesize oxidation tolerance mechanisms for lycopene biosynthesis are stronger in variety 1710 than in the other varieties, although future research is needed to confirm it.

The paste was supplemented with ascorbic acid, citric acid, sugar, and preservatives, in order to accomplish some quality standards and shelflife. Then, citric acid in all kinds of pasta is in the range of 11.88–15.76 mg AA, due to supplementation.

3.3. Total phenolic compounds (TPC) of the extracts

Fig. 3 (A–E) shows total phenols in tomato fruits and tissues. Tomatoes 115 and 117 did not show a significant difference in TPC during ripening; and JR and 1710 increased by 10–26% in G3 respecting G1 (4.6 vs 4.2, and 4.3 vs 3.4 mg GAE/g, respectively). Variety 1713 showed a small increase in G3, which decreased again in G6. Therefore, in general, concentrations of phenolics had little or no change during ripening. There was a moderate difference between TPC in the varieties of evaluated tomato: varieties 115 and 117 are in the range of 5.8–7.3 mg GAE/g (combining all fruit samples); and 1710, 1713, and JR were in the range of 2–5.6 mg GAE/g.

Phenolics in some leaves and stems had a notoriously significant difference, respecting fruit concentration. Stems of 117, 1710, 1713, and JR varieties had a lower concentration of phenolics than fruits (32-46% of fruit content), and similar levels in stems and fruits of 115. Contrarily, in some leaves, phenolics were higher than in their respective fruits. Specifically, in variety 115 total phenolics were 9.0 mg vs 6.3 mg GAE/g (leaves vs PH fruits, respectively), and in JR was 6.4 vs 5.6 mg GAE/g. In leaves of 117, 1710, and 1713, concentration is similar to or lower than in fruits. Our tomato fruits, stems, and leaves are in the range of 1.5-7.2 mg/g DW which was consistent with other tomato reports, e.g. TPC was found to be 2.680 \pm 0.107 mg/g in red tomato fruit L. cv. Cheers, from De Ruiter, France (Georgé et al., 2011); 2.541 \pm 0.089 mg/g in yellow tomato fruit L. cv. 6205, from Séminis, France (Georgé et al., 2011), 6.5911 ± 0.2328 mg/g in purple tomato fruit V118, from Ontario, Canada (Li et al., 2011), and 0.567 \pm 0.005 mg/g in cherry tomato fruit Mill. var. Moscatel RZ, from Portugal (Fernandes et al., 2021). TPC in varieties 115 and 117 is higher than several common vegetables such as beet (1.6941 \pm 0.4019 mg/g), potato (0.3842 \pm 0.0062 mg/g), carrots $(0.2221 \pm 0.0551 \text{ mg/g})$, or beans $(1.2941 \pm 0.1493 \text{ mg/g})$ (Sreeramulu and Raghunath, 2010). However, TPC in tomatoes is lower than rich-polyphenolic products such as green tea (31.6 \pm 0.31 mg/g) (Dereje, Minaleshewa, & Mirtachew, 2016), blackberry (104 \pm 1 mg/g) (Araya et al., 2017) or santol fruit 4004 ± 25 (Mesén-Mora, Álvarez-Valverde, Carvajal-Miranda, & Rodríguez-Rodríguez, 2019). According to literature grape extract with 4.430 \pm 0.017 mg GAE/g DW had been used to enrich pasta with phenolic compounds to 0.5-0.75 mg/g (Marinelli, Padalino, Nardiello, Del Nobile, & Conte, 2015). Then, either tomato byproducts or fruits were suitable to enrich antioxidants in processed starchy foods.

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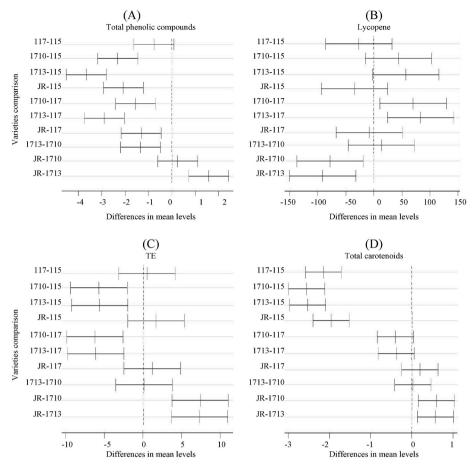


Fig. 4. Pairwise comparisons of differences in the mean level of tomato fruit samples at maturity G6, for: (A) Total phenolic compounds, (B) Lycopene, (C) TE & (D) Total carotenoids.

3.4. Antioxidant capacity of extracts

Lycopene and other carotenoids, ascorbic acid, diverse phenolic acids, and flavonoids are responsible for antioxidant capacity (García-Alonso et al., 2009), although we analyzed the ethanol-soluble fraction, mainly constituted by phenolic compounds. Fig. 3 (F-J) shows the antioxidant capacity of tomato fruits and tissues. Antioxidant capacity in tomato 115 stays at 22-23 TE/g for all ripening stages and post-harvest, but it decreases in the paste to 17.5 TE/g. Tomato 117 keeps very stable values during ripening (21-22 TE/g), as well, and slightly higher in post-harvest and salsa (26 and 24.5 TE/g respectively). In 1710 and 1713 varieties, antioxidant capacity fluctuates from 14 to 20 TE/g during ripening and post-harvest, with the maximum at G6 and G3, respectively. In both types of pastes, antioxidant levels decrease to 8-9 TE/g. The JR variety's antioxidant capacity is 18 TE/g during G1 and G3, but increases to 25.5 and 29.3 TE/g in G6 and post-harvest, respectively. It decreases again to 19 TE/g in paste, although, PH antioxidant capacity is 61% higher than G1-G3.

The antioxidant capacity of stems is approximately 30–50% lower than the corresponding fruits in all cases. Those values are consistent with phenolic concentrations. Also, some leaves, such as those from 115, 1710, 1713, and JR have a similar antioxidant capacity to their corresponding fruits.

3.5. Differences in antioxidant activity and metabolite mean levels between varieties

Fig. 4 shows the differences in mean levels for TPC, lycopene, antioxidant capacity, and total carotenoids in the G6 condition. The analysis compares pairwise combinations of tomato varieties. The TPC on varieties 115 and 117 (both for G6 conditions) were not significantly different (Fig. 4(a)). However, both 115, and 117 have higher TPC than all the other varieties included in this study. This behavior is not generalized for cherry-type tomatoes, respecting round-shape, or other cultivars (Bhandari, Cho, Lee, & Biotechnology, 2016). Although antioxidant activity has been determined in the ethanol extract (rich in TPC), TE is similar for either 115, 117, or JR. Those varieties have a higher TE than all the others included in this study. JR contains more Vitamin C than 115, and 117 (Fig. S1), and the combined effect of Vitamin C and TPC explains JR's antioxidant activity.

Varieties 1710 and 1713 have higher lycopene concentrations at G6 than all the other varieties, except 115 (Fig. 4(b)). On the other hand, variety 115 accounts for the highest total carotenoid concentration. There is no generalized trend between the concentrations of the antioxidant compounds in cherry and non-cherry tomatoes, but this is consistent with the literature (Bhandari, Cho, Lee, & Environment & Biotechnology, 2016). Although, higher lycopene concentration and selectivity have been observed in the autochthonous hybrid round-shaped fruits developed by the Fabio Baudrit Moreno Experimental Agronomic Station (varieties 1710, and 1713). These varieties probably are better acclimatized for production in tropical conditions.

3.6. Antibacterial activity of ethanolic extracts

The ethanolic extracts contain phenolic acids present in the tomatoes. Some phenolic secondary metabolites are well known for their antimicrobial properties. Table 2 shows the antimicrobial properties of the different extracts against 2 g-positive bacteria: Staphylococcus aureus, Bacillus subtilis, and 2 g-negative: Escherichia coli and Pseudomonas aeruginosa. All extracts show some activity against S. aureaus, except

Table 2Antimicrobial activity of ethanolic extracts of five varieties of tomato at three different ripening stages and post-harvest, against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Results are expressed as relative inhibition percent, using chloramphenicol as positive control.

Variety Ripening stage		% Relative Inhibition				
		Gram (+)		Gram (-)		
		S. aureus	B. subtilis	E. coli	P. aeruginosa	
115	G1	0	0	55±7 ^a	0	
	G3	0	0	0	0	
	G6	0	0	0	0	
	PH	0	0	0	0	
117	G1	40±3 ^a	$58,1\pm0,1^a$	69±4 ^a	0	
	G3	0	$51,6 \pm 0,1^{\mathrm{b}}$	0	$42,9 \pm 0,1^{a}$	
	G6	0	$58,1 \pm 0,1^{a}$	0	0	
	PH	0	0	0	0	
1710	G1	0	0	60±7ª	0	
	G3	$38,1 \pm 0,1^{\rm b}$	0	69 ± 4^a	$42,9 \pm 0,1^{a}$	
	G6	$42,9\pm0,1^a$	0	60 ± 7^a	0	
	PH	44±3 ^a	0	0	0	
1713	G1	40 ± 3^a	0	0	$38,1\pm0,1^{\rm a}$	
	G3	46±7 ^a	0	0	0	
	G6	$49,2\pm2,7^a$	$\textbf{45,2} \pm \textbf{0,1}^{\text{b}}$	0	0	
	PH	0	50±2a	0	0	
JR	G1	$42,9\pm0,1^a$	0	0	0	
_	G3	46±3 ^a	0	0	0	
	G6	42.9 ± 0.1^{a}	0	0	0	
	PH	44±3 ^a	0	0	0	

Letters in superscripts represent compact displays of Tukey's Honest Significant Difference. Tukey's analysis is independent for each variety.

variety 115. However, extracts from variety 117 show antimicrobial activity only in G1, and extracts from 1710 to 1713 do not show activity at G1 and PH, respectively. Extracts from 117 (excepting PH) and 1713 (excepting G1 and G3) are active against *B. subtilis*. Extracts G1 from 115 to 117 are active against *E. coli*, as well as G1, G2, and G3 from 1710. Finally, extracts G3, G3, and G1 from varieties 117, 1710, and 1713, specifically, are antimicrobial against *P. aeruginosa*.

Interestingly, varieties 115 and 117, both "cherry" types, show antimicrobial properties against some bacterial strains at the early ripening stages, and this capacity seems to be lost in ripe fruits. On the other hand, the ripening process usually does not change the antimicrobial capacity for round-shaped varieties (1710, 1713, and JR), with a few exceptions. Round-shaped tomatoes are inhibitors for *S. aureaus*. Extract from variety 117 has a wide range of antimicrobial capacity when it becomes from immature fruits, but it is lost with ripening (Table 2).

4. Conclusions

The concentrations of lycopene, total carotenoids, vitamin C and phenolic compounds are highly dependent on the analyzed variety. Tomato 1710 has the potential for the production of lycopene-rich pastes because it does not present a severe decay in the concentration of this metabolite during the processing, while all the other varieties suffered an important degradation of lycopene during paste processing. This behavior offers an advantage for lycopene extraction purposes, because the more oxidative sensitivity, the milder conditions are needed. Also, better functional food products can be prepared from this variety. On the other hand, the JR variety shows the highest lycopene content on the shelves, but it suffers an important degradation during processing.

Lycopene and total carotenoids increase during tomato ripening.

Vitamin C keeps high levels during all ripening stages. Phenolic compounds of tomato fruits suffer low or no change with ripening and processing. A few exceptions suffer a decay of phenolics during processing, such as variety 117. Tomato leaves are comparable to fruits in terms of phenolic compounds and antioxidant activity of all varieties, except tomato 117. Stems are up to 68% lower in phenolics (and antioxidant activity) than fruits, but they still can be used as a good source of those components, considering they are sub-products of agricultural activity.

Some ethanolic extracts from tomatoes contain antimicrobial activity. The activity is more frequent in the early ripening stages for cherry-type tomatoes. All round-shape varieties included in this study were active against the gram-positive *Staphylococcus aureus*.

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CRediT authorship contribution statement

Brainer Vega-López: Methodology, Investigation, Formal analysis. Yendry Carvajal-Miranda: Funding acquisition, Supervision. Laura Brenes-Peralta: Funding acquisition, Supervision, Writing – review & editing. Marianela Gamboa-Murillo: Funding acquisition, Supervision. Jimmy Venegas-Padilla: Data curation, Formal analysis. Gerardo Rodríguez: Funding acquisition, Project administration, Supervision. Pablo Jiménez-Bonilla: Visualization, Formal analysis, Writing – original draft. Victor Álvarez-Valverde: Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Abushita, A., Daood, H., & Biacs, P. (2000). Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry*, 48(6), 2075–2081.

Araya, M., Carvajal, Y., Alvarez, V., Orozco, R., & Rodriguez, G. (2017). Polyphenol characterization of three varieties of blackberry fruits (*Rubus adenotrichos*), cultivated in Costa Rica. *Journal of Berry Research*, 7(2), 97–107.

Baldwin, E. A., Scott, J. W., Shewmaker, C. K., & Schuch, W. (2000). Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *HortScience*, 35(6), 1013–1022.

Bhandari, S. R., Cho, M.-C., Lee, J. G. J. H., & Environment, & Biotechnology. (2016). Genotypic variation in carotenoid, ascorbic acid, total phenolic, and flavonoid contents, and antioxidant activity in selected tomato breeding lines. *Horticulture, Environment, and Biotechnology, 57*(5), 440–452.

- Bui, H.-T., Makhlouf, J., & Ratti, C. (2010). Postharvest ripening characterization of greenhouse tomatoes. *International Journal of Food Properties*, 13(4), 830–846.
- Demiray, E., Tulek, Y., & Yilmaz, Y. (2013). Degradation kinetics of lycopene, β-carotene and ascorbic acid in tomatoes during hot air drying. *LWT-Food Science and Technology*, 50(1), 172–176.
- Dereje, B., Minaleshewa, A., & Mirtachew, T. (2016). Determination of some selected secondary metabolites and their in vitro antioxidant activity in commercially available Ethiopian tea (Camellia sinensis). SpringerPlus, 5, 412. https://doi.org/ 10.1186/s40064-016-2056-1
- FAO. (2019). FAOSTAT statistical database. from FAO http://www.fao.org/faostat/en/#data.
- Farooq, S., Rather, A., Gull, A., Ahmad Ganai, S., Masoodi, F., Mohd Wani, S., et al. (2020). Physicochemical and nutraceutical properties of tomato powder as affected by pretreatments, drying methods, and storage period. *International Journal of Food Properties*. 23(1), 797–808.
- Fernandes, I., Leça, J. M., Aguiar, R., Fernandes, T., Marques, J. C., & Cordeiro, N. (2021). Influence of crop system fruit quality, carotenoids, fatty acids and phenolic compounds in cherry tomatoes. Agricultural Research, 10(1), 56–65.
- García-Alonso, F. J., Bravo, S., Casas, J., Perez-Conesa, D., Jacob, K., & Periago, M. J. (2009). Changes in antioxidant compounds during the shelf life of commercial tomato juices in different packaging materials. *Journal of Agricultural and Food Chemistry*, 57(15), 6815–6822.
- Georgé, S., Tourniaire, F., Gautier, H., Goupy, P., Rock, E., & Caris-Veyrat, C. (2011). Changes in the contents of carotenoids, phenolic compounds and vitamin C during technical processing and lyophilisation of red and yellow tomatoes. *Food Chemistry*, 124(4), 1603–1611.
- Li, H., Deng, Z., Liu, R., Young, J. C., Zhu, H., Loewen, S., et al. (2011). Characterization of phytochemicals and antioxidant activities of a purple tomato (Solanum lycopersicum L.). Journal of Agricultural and Food Chemistry, 59(21), 11803–11811.
- Lopez-Marín, L. (2017). Manual técnico de Tomate. San José, Costa Rica: INTA. Lopez-Marín, L., Brenes-Peralta, L., Jiménez-Morales, M. F., & Gonzalez-Masis, M.
- Lopez-Marín, L., Brenes-Peralta, L., Jimenez-Morales, M. F., & Gonzalez-Masis, M. (2019). Costos de produccion, valor agregado y pérdidas de tomate. Cartago, Costa Rica. Editorial Tecnológica.
- Manzo, N., Santini, A., Pizzolongo, F., Aiello, A., & Romano, R. (2019). Degradation kinetic (D100) of lycopene during the thermal treatment of concentrated tomato paste. *Natural Product Research*, 33(13), 1835–1841.
- Martínez-Hernández, G. B., Boluda-Aguilar, M., Taboada-Rodríguez, A., Soto-Jover, S., Marín-Iniesta, F., & López-Gómez, A. (2016). Processing, packaging, and storage of tomato products: Influence on the lycopene content. Food Engineering Reviews, 8(1), 52–75.
- Marinelli, V., Padalino, L., Nardiello, D., Del Nobile, M. A., & Conte, A. (2015). New approach to enrich pasta with polyphenols from grape marc. *Journal of Chemistry*, 2015, Article 734578. https://doi.org/10.1155/2015/734578
- Martí, R., Roselló, S., & Cebolla-Cornejo, J. (2016). Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *Cancers*, 8(6), 58.
- Mesén-Mora, L. D., Álvarez-Valverde, V., Carvajal-Miranda, Y., & Rodríguez-Rodríguez, G. (2019). Bioprospecting study, antibiotic and antioxidant activity of the santol's fruit. Sandoricum koetjape) Uniciencia, 33(1), 75–82.

- Monge-Pérez, J. (2014). Caracterización de 14 genotipos de tomate (Lycopersicon esculentum Mill.) cultivados bajo invernadero en Costa Rica. Tecnología En Marcha, 27(4), 58–68. https://doi.org/10.18845/tm.v27i4.2086
- OFINASE. (2021). Register of commercial seed varieties. Retrieved from http://ofinase.go.cr/servicios/variedadescomerciales/.
- R Core Team. (2021). R: A language and environment for statical computing. Vienna,
 Austria: R Foundation for Statical Computing. Retrieved from https://www.
 R-project.
- Ronen, G., Cohen, M., Zamir, D., & Hirschberg, J. (1999). Regulation of carotenoid biosynthesis during tomato fruit development: Expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant delta. The Plant Journal, 17(4), 341–351.
- Saini, R. K., Rengasamy, K. R., Mahomoodally, F. M., & Keum, Y.-S. (2020). Protective effects of lycopene in cancer, cardiovascular, and neurodegenerative diseases: An update on epidemiological and mechanistic perspectives. *Pharmacological Research*, 155, 104730.
- Sapei, L., & Hwa, L. (2014). Study on the kinetics of vitamin C degradation in fresh strawberry juices. *Procedia Chemistry*, 9, 62–68.
- Shibata, S., Ishihara, C., & Matsumoto, K. (2004). Improved separation method for highly purified lutein from chlorella powder using jet mill and flash column chromatography on silica gel. *Journal of Agricultural and Food Chemistry*, 52(20), 6283–6286.
- Shi, J., Kakuda, Y., & Yeung, D. (2004). Antioxidative properties of lycopene and other carotenoids from tomatoes: Synergistic effects. BioFactors, 21(1-4), 203-210.
- Shi, J., & Maguer, M. L. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. Critical reviews in food science, 40(1), 1–42.
- Sreeramulu, D., & Raghunath, M. (2010). Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. Food Research International, 43(4), 1017–1020.
- Stevens, R., Buret, M., Garchery, C., Carretero, Y., & Causse, M. (2006). Technique for rapid, small-scale analysis of vitamin C levels in fruit and application to a tomato mutant collection. *Journal of Agricultural and Food Chemistry*, 54(17), 6159–6165.
- Syedd-León, R., Orozco, R., Álvarez, V., Carvajal, Y., & Rodríguez, G. (2020). Chemical and antioxidant characterization of native corn germplasm from two regions of Costa Rica: A conservation approach. *International journal of food science*, 2020.
- Tamasi, G., Pardini, A., Bonechi, C., Donati, A., Pessina, F., Marcolongo, P., et al. (2019). Characterization of nutraceutical components in tomato pulp, skin and locular gel. European Food Research and Technology, 245(4), 907–918.
- Tatsuzawa, H., Maruyama, T., Misawa, N., Fujimori, K., & Nakano, M. (2000). Quenching of singlet oxygen by carotenoids produced in *Escherichia coli*—attenuation of singlet oxygen-mediated bacterial killing by carotenoids. *FEBS Letters*, 484(3), 280–284.
- Uddin, M., Hawlader, M., & Zhou, L. (2001). Kinetics of ascorbic acid degradation in dried kiwifruits during storage. *Drying Technology*, 19(2), 437–446.
- USDA. (1991). U.S. Grade standard for tomatoes. Retrieved from https://www.ams.usda.gov/grades-standards/tomato-grades-and-standards.
- Zhang, Y., Li, Z., Tu, Y., Cheng, W., & Yang, Y. (2018). Tomato (Solanum lycopersicum) SIIPT4, encoding an isopentenyltransferase, is involved in leaf senescence and lycopene biosynthesis during fruit ripening. BMC Plant Biology, 18(1), 1–12.