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ORIGINAL ARTICLE

Metabolic changes associated with chilling injury tolerance in tomato fruit with hot water pretreatment

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Abstract

Hot water treatment (HWT) of tomato (*Solanum lycopersicum* L.) fruit reduces the symptoms of chilling injury (CI). The aim of this study was to identify metabolites associated with HWT-induced CI tolerance in tomato fruit cv. Imperial. Mature green tomatoes with HWT (42°C/5 min) and control were stored under chilling conditions (5°C/20 days) and then ripened (21°C/7 days). Methanol extracts from pericarp were analyzed for total phenolics (TP), antioxidant activity (AoxA), and metabolic profiling by UPLC-DAD-MS and GC-MS. After cold storage and ripening, HWT fruit showed less CI, higher TP, and AoxA than control. It also showed an increased accumulation of phenolics, sugars, and some alkaloids that may be mediated by azelaic acid, glutamine, and tryptophan. The levels of *N*-feruloyl putrescine, esculeoside AII, and hydroxy-α-tomatine II were reduced. The better metabolic performance of HWT fruit under cold storage was associated with a higher accumulation of several metabolites (e.g., antioxidants and osmolytes) in ripening fruit.

Practical application: The identification of metabolites associated with the reduction of chilling injury (CI) symptoms in HWT tomato fruit extends the understanding of the mechanisms involved in CI tolerance. This information provides targets that could be used to develop strategies for preventing CI (e.g., genetic improvement of tomato, direct application of key metabolites). The application of such strategies will increase the economic value and decrease postharvest losses.

KEYWORDS
chilling tolerance, hot water treatment, metabolomics, tomato

1 | INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important fruit worldwide due to its high consumption in fresh and processed. This fruit is usually harvested at the mature green stage and stored at low temperatures to extend its shelf life; however, it is susceptible to chilling injury (CI) when exposed to temperatures below 10–13°C (Aghdam & Bodbodak, 2014). CI decreases fruit quality, and the main symptoms include uneven ripening, surface pitting, and decay (Vega-Garcia et al., 2010). The mechanisms involved in the CI disorder are poorly understood. However, the phase transition of the cell membrane lipids has been considered the primary response to cold stress (Lyons, 1973). In cold stressed plants, an oxidative burst has been registered and oxidative stress response has also been associated with the development of CI symptoms, suggesting that the antioxidant system confers protection to the tissues against chilling stress (Giordano et al., 2021; Gonzalez et al., 2019; Zhang et al., 2019).

Omics studies have provided information about the mechanisms associated with the response of plants to cold stress. Metabolomic analysis of Micro-Tom tomato fruit by GC-MS showed that chilling changes the profile of key metabolites of glycolysis and pentose phosphate pathway (i.e., glucose-6-phosphate and fructose-6-phosphate), increases the levels of arabinose, citric acid, dehydroascorbic acid, and valine; and it decreases the levels of glutamic and shikimic acids (Luengwilai, Saltveit, et al., 2012). NMR studies of the effect of cold stress in tubers of five potato cultivars showed evidence that the cold response is associated with adjustments in the biochemical pathways of carbohydrates, amino acids, and organic acids (Datir et al., 2020). Transcriptome and metabolome analyses showed that putrescine is important in the cold tolerance of the potato tuber of *Solanum acaule*, showing also an accumulation of saccharides, amino acids, γ-aminobutyric acid (GABA), among other metabolites (Kou et al., 2018). Albornoz et al. (2019) showed the importance of the metabolism of ethylene and carbohydrates in the response to cold stress of cherry tomatoes. The analysis of the transcriptome and metabolome of tomato cv. Defunis evidenced an important role of the ethylene and flavonoid metabolism in cold response (Park et al., 2021). In Micro-Tom tomato, metabolome analysis showed that fermentative metabolism and accumulation of amino acids (e.g., glutamic acid and glutamine) and GABA are activated by cold stress. Furthermore, it was demonstrated that cold stress downregulates genes of photosynthesis, Calvin cycle, and pigment biosynthesis, processes that were activated by removing the fruit from cold stress (Gonzalez et al., 2019). Another study in tomato fruit showed that cold stress induced the metabolism reprogramming: for example, the biosynthesis of amino acids, flavonoids, and pentose phosphate pathway is induced, whereas variations in the content of organic acids and sugars, depending on the time and storage condition, are described (Zhang et al., 2019).

Some treatments used to induce CI tolerance in tomato fruit include the application of hot water treatment (HWT) (Cárdenas-Torres et al., 2020; Luengwilai, Beckles, et al., 2012), melatonin (Jannatizadeh et al., 2019), carbon dioxide (Park et al., 2021), and phenylalanine (Aghdam et al., 2019). HWT is relatively easy to apply at a low cost. The CI tolerance induced by HWT in tomato (cv. Imperial) has been associated with changes in the expression of genes encoding heat shock proteins and enzymes of the antioxidant system and the energy metabolism (Cruz-Mendivil et al., 2015; Salazar-Salas et al., 2017); however, the metabolites associated with such tolerance have not been identified. In this regard, the CI tolerance induction has been associated with a range of metabolites and depends on the species, varieties of the same species, and developmental stage, among other factors (Aghdam et al., 2019; Albornoz et al., 2019; Datir et al., 2020; Gonzalez et al., 2019; Jannatizadeh et al., 2019; Loayza et al., 2021; Luengwilai, Saltveit, et al., 2012; Zhang et al., 2019).

The aim of the present study was to analyze changes in the metabolome of tomato (cv. Imperial) pericarp in response to HWT, cold storage, and ripening to gain more insight into the mechanisms of CI tolerance induced by HWT.

2 | MATERIALS AND METHODS

2.1 | Plant material and postharvest treatments

Mature green tomato fruit (*Solanum lycopersicum* cv. Imperial) were obtained from a local producer and selected based on size uniformity, color, and absence of defects. Ninety fruit were washed (sodium hypochlorite solution, 200 mg/L) and divided into two groups: one group (45 fruits) was used as control and the other group (45 fruits) was immersed in hot water at 42°C for 5 min. The HWT conditions for this tomato cultivar were previously established based on the effectiveness of the treatment to provide CI tolerance as evidenced by physiological, biochemical, and molecular data (Cárdenas-Torres et al., 2020; Salazar-Salas et al., 2017). Control and HWT fruit were stored sequentially under the following three conditions: 0 days at 5°C, 20 days at 5°C (80%–90% relative humidity), and 7 days at 21°C (80% relative humidity). Fifteen fruit per treatment (three replicates of five fruits) were removed after each storage condition for chilling injury (CI) index. After CI analysis, fruits were cut along the equator to remove the seeds and the pulp with a small spoon. The pericarp (flesh) was frozen in liquid nitrogen, lyophilized, ground to a fine powder, and stored at −70°C until use for metabolite analyses.

2.2 | Chilling injury index (CII)

This index was measured according to Vega-Garcia et al. (2010). The symptoms evaluated were uneven ripening (U), shriveling (S), pitting (P), and decay (D). The severity of each symptom was visually assessed on each fruit as injury level (IL) using a 5-point scale based on the percentage of tissue affected (0 = no injury, 1 = ≤10%, 2 = 11%–25%, 3 = 26%–40%, 4 = >40%). The IL values of the four symptoms were averaged to calculate the extent of damage for each fruit: CII = (ILU + ILS + ILP + ILD)/4. The CII value reported for each treatment/storage condition is an average of 15 fruit (five per replicate).

2.3 | Preparation of methanol extracts

Methanol extracts were obtained according to Gómez-Romero et al. (2010) with some modifications. Lyophilized pericarp (0.5 g) was mixed with 5 mL of methanol, sonicated for 15 min, centrifuged (28,620 × g/15 min/20°C), and the supernatant was recovered. The extraction was repeated two times with the residue. The supernatants were mixed and then dried under vacuum (40°C) with a rotary evaporator (BÜCHI R-124, Brinkmann Instruments, USA). The extract was resuspended in 2 mL of methanol and passed through a syringe filter (PVDF membrane, 0.45 μm, HPLC certified, Thermo Scientific, Germany).

2.4 | Total phenolics (TP)

TP content was determined by the Folin-Ciocalteu assay (Waterhouse, 2002). A 20-μL aliquot of methanol extract was mixed with 1.58 mL of deionized water and 0.1 ml of the Folin-Ciocalteu reagent, gently stirred for 5 min, and added with 0.3 mL of a sodium carbonate saturated solution. The mixture was incubated in darkness (30 min at 40°C) and the absorbance was registered at 765 nm. A gallic acid standard curve was prepared, and the results were reported as grams of Gallic Acid Equivalents (GAE) per kg on a fresh weight basis (g GAE/kg fw).

2.5 | Antioxidant activity (AoxA)

The AoxA of the methanol extracts was measured by two methods: 2,2′-azino-bis(3-ethylbenzothiazolin)-6-sulfonic acid (ABTS) (Re et al., 1999) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995). It was expressed as millimoles of Trolox Equivalents per kilogram of fresh weight (mmol TE/kg fw). For ABTS, 0.05 mL of methanol extract or methanol (blank) was mixed with 1.95 mL of the $ABTS^{\bullet+}$ radical solution (5 mL of 7 mmol/L ABTS and 0.088 mL of 140 mmol/L sodium persulfate) in a microplate, incubated at 37°C for 10 min and then the absorbance was registered at 734 nm (Multiskan Sky, Thermo Fisher, USA). For DPPH, 0.2 mL of the methanol extract or methanol (blank) was mixed with 1.8 mL of 0.15 mmol/L DPPH in methanol; the mixture was incubated in darkness for 20 min at room temperature, and the absorbance was read at 515 nm.

2.6 | Metabolic profiles by Ultra Performance Liquid Chromatography-Diode Array Detection and Mass Spectrometry analysis (UPLC-DAD-MS)

UPLC analysis was performed according to Moco et al. (2006) with some modifications. A 5-μL aliquot of the methanol extract was analyzed using an UPLC-DAD system (ACCELA, Thermo Scientific, USA). The column used was a Fortis C18 HPLC (3 μm, 50 × 2.1 mm) (Fortis Technologies Ltd, UK) with a flow rate of 0.2 mL/min of 1% (v/v) formic acid (A) and acetonitrile (B), using a linear gradient from 0.5% to 60% of B in 40 min. The detection was done at 280, 320, and 350 nm. The internal standards were protocatechuic acid and daidzein. The identification of the compounds was based on the UV-spectra, MS fragmentation, and MS data from commercial standards and scientific reports (Gómez-Romero et al., 2010; Moco et al., 2006). The quantification was conducted using calibration curves: phenolic acids (chlorogenic, caffeic, ferulic, *p*-coumaric, and syringic), flavonoids (rutin, quercetin, kaempferol, and naringenin), alkaloids (tomatidine), and others (tryptophan, abscisic acid, and azelaic acid) (Sigma Chemical Co., St. Louis, MO, USA). The results were expressed in milligrams per kilogram of sample on a fresh weight basis (mg/kg fw). The UPLC-DAD was coupled to a mass spectrometer with an electrospray ionization source (ESI) (LTQ XL, Thermo Scientific, USA). Full scan spectra (negative mode) were obtained in the *m/z* range of 110–2000. Some ions were used for MS^n fragmentation by collision-induced dissociation applying 10–45 V. The capillary tube was set at 35 V and 300°C. Nitrogen and helium gases were used for drying and collision, respectively. The results were analyzed with the Xcalibur 2.2 software (Thermo Scientific, USA).

2.7 | Metabolic profiles by Gas Chromatography and Mass Spectrometry analysis (GC-MS)

The methanol extracts were derivatized according to Wang and Zuo (2011) with some modifications. A 50-μL aliquot of the filtered methanol extract was transferred into a vial, dried with a stream of N_2 gas, mixed with 100 μL of pyridine, and 100 μL of BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) + 1% TMSCI (trimethylsilyl chloride) and incubated at 70°C for 3 hr. The resulting mixture was analyzed using an HP 6890 capillary GC system coupled to an Agilent 5973N mass spectrometer (Agilent Technologies, USA). The compounds were separated in a QUADREX 007 CARBOWAX 20 M column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature program was as follows: 60°C for 1 min, 60–200°C at 5°C/min, 200–275°C at 10°C/min, 275°C for 10 min. The temperatures of the injector and the interphase were 250 and 300°C, respectively. The carrier gas (helium) flow was 0.9 mL/min. The mass spectrometer operated in electron impact (EI) mode at 70 eV in the full-scan mode in the 50–800 amu range. The derivatized sample was injected (5 μL) by an autosampler in the split mode. The identification of the compounds was done by comparison of the mass spectra and retention time obtained from authentic standards (lactic acid, fumaric acid, malic acid, isocitric acid, isoleucine, glycine, serine, aspartic acid, phenylalanine, fructose, glucose, and sucrose), and the mass spectra available in the NIST08 library. The area of each peak was normalized using rhamnose and tryptophan as internal standards. To obtain the relative content, the normalized area of each compound was divided by that of the same compound in the control fruit before cold storage (0 d at 5°C).

2.8 | Statistical analysis

Data were analyzed by two-way ANOVA to test for significant differences between the treatments and storage conditions. The values are the mean of three replicates and were compared by the least significant difference (LSD) test at $p \leq .05$. Pearson correlation analysis between total phenolics, phenolic compounds,

and antioxidant activity was carried out. All analyses were performed using STATGRAPHIC plus version 5.1 (Statistical Graphics Corporation™, Maryland, USA).

3 | RESULTS

3.1 | Chilling injury index (CII), total phenolics (TP) content, and antioxidant activity

CII values showed that chilling injury was significantly higher in control than HWT fruit after 20 days of cold storage (5°C) plus the ripening period (Table 1). Analyzing the CI symptoms, uneven ripening, pitting, shriveling, and decay were mainly observed in the control fruit (Figure S1), whereas the HWT fruit showed a more uniform ripening and less incidence of decay.

TP content in the control fruit did not change significantly after cold storage and ripening. On the contrary, HWT fruit showed almost a 50% increase in TP content after 20 days at 5°C; this value decreased after ripening but it was still significantly higher than that of the control fruit (Table 1). In the case of the antioxidant activity, there was a general increase in ABTS and DPPH values after the cold storage, and values for control and HWT fruit were statistically similar. After ripening, both antioxidant values were significantly higher in HWT than in control fruit. A correlation between TP and antioxidant activity was only found after ripening (DPPH: $r = 0.88$, $p \leq .05$; ABTS, $r = 0.95$, $p \leq .01$). These results suggested that antioxidants such as phenolics may contribute to the HWT-induced chilling injury tolerance in tomatoes. Thus, metabolic profiles were obtained to investigate the specific compounds that may be contributing to this tolerance.

3.2 | Changes in the abundance of secondary metabolites in response to hot water treatment, cold storage, and ripening

UPLC analysis separated 22 compounds (Figure S2), which included mainly phenolic acids and flavonoids, as well as some alkaloids and other metabolites (one amino acid, one terpene, and one organic acid) (Table S1). The compounds with the highest concentration in the control fruit before cold storage were azelaic acid, naringenin chalcone, chlorogenic acid, and tryptophan (Table 2).

The abundance of compounds in the tomato pericarp was significantly affected by the HWT and the storage conditions. The HWT applied before cold storage (0 days at 5°C) induced significant variations in the abundance of 13 metabolites, 10 of them increased their content (e.g., 5-caffeoylquinic acid, dehydrolycoperoside I, azelaic acid) and three decreased their levels: tricaffeoylquinic acid, chlorogenic acid (3-caffeoylquinic acid), and tryptophan (Tables 2 and S2). The data suggest that the HWT induced chlorogenic acid isomerization to 5-caffeoylquinic acid (Table 2). After cold storage, the levels of most metabolites decreased, showing lower values in HWT than control fruit. In both treatments, there was a significant increase in the levels of *N*-feruloyl putrescine, esculeoside AII, and hydroxy-α-tomatine II, while the contents of coumaroyl-hexose, caffeoyl-hexose I, and kaempferol-3-*O*-rutinoside increased significantly only in control fruit (Tables 2 and S2). After cold storage and ripening, the levels of most metabolites increased and their values were higher in HWT than in control fruit. However, lower values in both control and HWT fruit were registered for syringic acid, rutin pentoside, and quercetin-hexose-deoxyhexose-pentose-*p*-coumaric acid. Control fruit also showed a lower value for caffeic acid (Tables 2 and S2). During ripening, the control fruit maintained

TABLE 1 Chilling injury index, total phenolics content, and antioxidant activity of control and HW-treated tomato fruit stored under chilling conditions and ripened

Treatment	Storage condition		
	0 days at 5°C	20 days at 5°C	20 days at 5°C + 7 days at 21°C
Chilling injury index			
Control	ND	ND	1.65^{a}
HW	ND	ND	0.57^{b}
Total phenolics (g GAE/kg)			
Control	0.29 ± 0.04^{Aa}	0.31 ± 0.02^{Ab}	0.26 ± 0.01^{Ab}
HW	0.30 ± 0.04^{Ba}	0.44 ± 0.05^{Aa}	0.33 ± 0.01^{Ba}
ABTS (mmol TE/kg)			
Control	6.10 ± 0.53^{Ba}	8.25 ± 0.57^{Aa}	5.14 ± 0.06^{Cb}
HW	6.16 ± 0.31^{Ba}	8.40 ± 0.66^{Aa}	6.51 ± 0.46^{Ba}
DPPH (mmol TE/kg)			
Control	2.90 ± 0.23^{Bb}	3.66 ± 0.06^{Aa}	2.56 ± 0.20^{Bb}
HW	3.34 ± 0.15^{Aa}	3.43 ± 0.31^{Aa}	3.30 ± 0.24^{Aa}

Note: Values are the mean ± *SD* of three replicates. Different letters in the same row (A, B, and C) or column (a and b) indicate significant differences between storage conditions and treatments, respectively (Fisher, $\alpha = 0.05$).

Abbreviations: GAE, gallic acid equivalents; HW, hot water; TE, trolox equivalents.

TABLE 2 Content of metabolites identified by UPLC-DAD-MS in the pericarp of control and HW-treated "Imperial" tomato fruit after cold storage and ripening

Metabolite	Treatment	Storage condition			LOD
		0 days at 5°C	20 days at 5°C	20 days at 5°C + 7 days at 21°C	
Phenolics (mg/kg)					
Coumaroyl-hexose†	Control	1.88 ± 0.23^{Bb}	2.70 ± 0.03^{Aa}	2.62 ± 0.12^{Aa}	0.0003
	HW	2.89 ± 0.12^{Aa}	1.80 ± 0.21^{Bb}	2.90 ± 0.34^{Aa}	
Caffeoyl hexose I†	Control	0.61 ± 0.03^{Ba}	2.04 ± 0.44^{Aa}	1.98 ± 0.29^{Ab}	0.0008
	HW	0.39 ± 0.04^{Ba}	0.89 ± 0.01^{Bb}	4.90 ± 0.22^{Aa}	
Caffeic acid	Control	4.48 ± 0.74^{Aa}	4.99 ± 0.05^{Aa}	2.82 ± 0.47^{Bb}	0.0008
	HW	3.55 ± 1.12^{ABa}	2.50 ± 0.03^{Bb}	4.23 ± 1.07^{Aa}	
Chlorogenic acid	Control	54.34 ± 11.18^{Aa}	8.14 ± 0.59^{Ba}	8.20 ± 0.92^{Ba}	0.0041
	HW	36.25 ± 8.12^{Ab}	8.66 ± 0.54^{Ba}	15.57 ± 1.03^{Ba}	
5-Caffeoylquinic acid	Control	6.71 ± 1.38^{Ab}	0.89 ± 0.28^{Ba}	3.13 ± 0.07^{Bb}	0.0041
	HW	13.69 ± 3.22^{Aa}	0.78 ± 0.19^{Ca}	6.12 ± 0.99^{Ba}	
Tricaffeoylquinic acid†	Control	0.30 ± 0.01^{Ba}	0.30 ± 0.09^{Ba}	0.82 ± 0.08^{Aa}	0.0008
	HW	0.06 ± 0.04^{Ab}	0.08 ± 0.01^{Ab}	0.14 ± 0.05^{Ab}	
N-Feruloyl putrescine†	Control	ND	2.96 ± 0.05^{Ba}	3.43 ± 0.31^{Ab}	0.0007
	HW	ND	2.22 ± 0.09^{Bb}	3.88 ± 0.07^{Aa}	
Ferulic acid	Control	0.86 ± 0.07^{Ba}	1.01 ± 0.09^{Ba}	2.67 ± 0.34^{Ab}	0.0007
	HW	1.11 ± 0.36^{Ba}	0.78 ± 0.05^{Ba}	3.19 ± 0.18^{Aa}	
Syringic acid	Control	6.29 ± 0.21^{Aa}	3.55 ± 0.41^{Ba}	ND	0.0007
	HW	6.96 ± 1.74^{Aa}	3.18 ± 0.39^{Ba}	ND	
Naringenin chalcone†	Control	58.52 ± 6.68^{Ab}	7.71 ± 2.36^{Ba}	9.16 ± 0.11^{Ba}	0.0022
	HW	67.41 ± 5.53^{Aa}	3.61 ± 0.21^{Ba}	9.71 ± 0.62^{Ba}	
Kaempferol-3-*O*-rutinoside†	Control	0.63 ± 0.08^{Cb}	0.86 ± 0.06^{Ba}	1.06 ± 0.06^{Ab}	0.0003
	HW	1.41 ± 0.15^{Aa}	0.66 ± 0.07^{Bb}	1.35 ± 0.12^{Aa}	
Rutin pentoside†	Control	3.08 ± 0.25^{Ab}	3.20 ± 0.07^{Aa}	1.81 ± 0.30^{Bb}	0.0006
	HW	5.09 ± 0.39^{Aa}	2.98 ± 0.36^{Ba}	2.38 ± 0.12^{Ca}	
Rutin	Control	3.16 ± 0.43^{Ab}	1.58 ± 0.10^{Bb}	1.20 ± 0.26^{Bb}	0.0006
	HW	5.23 ± 0.33^{Aa}	2.27 ± 0.31^{Ba}	2.60 ± 0.22^{Ba}	
Quercetin-hexose-deoxyhexose-pentose-p-coumaric acid†	Control	1.74 ± 0.30^{Aa}	1.03 ± 0.05^{Ba}	ND	0.0001
	HW	1.49 ± 0.05^{Aa}	0.85 ± 0.01^{Ba}	ND	
Alkaloids (mg/kg)					
Dehydrolycoperoside‡ + [FA]	Control	9.32 ± 1.70^{Bb}	11.64 ± 2.36^{Ba}	25.96 ± 0.56^{Aa}	0.0030
	HW	31.28 ± 4.46^{Aa}	7.38 ± 0.58^{Ba}	29.49 ± 3.15^{Aa}	
Dehydrolycoperoside‡ + [FA]	Control	13.88 ± 1.50^{Ab}	3.91 ± 1.00^{Ba}	4.65 ± 0.52^{Bb}	0.0030
	HW	25.64 ± 2.80^{Aa}	2.67 ± 0.49^{Ca}	10.55 ± 0.33^{Ba}	
Total	Control	23.20 ± 3.21^{Bb}	15.56 ± 2.76^{Ca}	30.62 ± 1.05^{Ab}	
	HW	56.92 ± 7.26^{Aa}	10.55 ± 0.93^{Ca}	40.04 ± 3.48^{Ba}	
Esculeoside A I‡	Control	13.10 ± 1.99^{Ab}	5.61 ± 0.48^{Ba}	4.86 ± 0.80^{Bb}	0.0030
	HW	23.50 ± 2.45^{Aa}	3.93 ± 0.64^{Ca}	10.74 ± 0.76^{Ba}	
Esculeoside A II‡	Control	ND	10.18 ± 1.04^{Aa}	9.18 ± 1.08^{Ab}	0.0030
	HW	ND	6.83 ± 0.38^{Bb}	15.55 ± 1.30^{Aa}	
Total	Control	13.10 ± 1.99^{Ab}	15.79 ± 1.44^{Aa}	14.04 ± 0.60^{Ab}	
	HW	23.50 ± 2.45^{Aa}	10.76 ± 0.77^{Bb}	26.30 ± 2.06^{Aa}	

(Continues)

TABLE 2 (Continued)

Metabolite	Treatment	Storage condition			LOD
		0 days at 5°C	20 days at 5°C	20 days at 5°C + 7 days at 21°C	
Hydroxy-α-Tomatine II‡ + [FA]	Control	ND	3.66 ± 0.42^{Ba}	5.91 ± 0.31^{Aa}	0.0030
	HW	ND	1.44 ± 0.38^{Bb}	4.57 ± 0.29^{Ab}	
Other metabolites (mg/kg)					
Tryptophan + [NH4+]	Control	16.75 ± 1.00^{Aa}	8.73 ± 0.82^{Cb}	12.14 ± 0.69^{Bb}	0.0060
	HW	12.47 ± 0.97^{Bb}	13.26 ± 0.26^{ABa}	15.08 ± 1.55^{Aa}	
Dehydrophaseic acid-hexose§	Control	1.00 ± 0.11^{Aa}	0.50 ± 0.05^{Ca}	0.72 ± 0.05^{Bb}	0.0004
	HW	1.06 ± 0.07^{Ba}	0.55 ± 0.01^{Ca}	1.44 ± 0.17^{Aa}	
Azelaic acid	Control	195.94 ± 12.94^{Ab}	192.2 ± 12.48^{Aa}	212.85 ± 17.02^{Ab}	0.0799
	HW	243.03 ± 19.79^{Ba}	151.72 ± 6.85^{Cb}	306.77 ± 26.77^{Aa}	

Note: Values are the mean ± *SD* of three replicates and are expressed on a fresh weight basis.

Different letters in the same row (A, B, and C) or column (a and b) indicate significant differences (Fisher, $p < .05$) between storage conditions and treatments, respectively.

Abbreviations: HW, hot water; LOD, limit of detection (mg/mL); ND, not detected.

†mg equivalents of the aglycone/kg.

‡mg equivalents of tomatidine/kg.

§mg equivalents of abscisic acid/kg.

high levels of coumaroyl-hexose, caffeoyl-hexose I, and kaempferol-3-*O*-rutinoside; but it showed increased levels of ferulic acid, tri-caffeoylquinic acid, *N*-feruloyl putrescine, dehydrolycoperoside I, hydroxy-α-tomatine II, tryptophan, and dehydrophaseic acid hexose (Tables 2 and S2). On the other hand, the HWT fruit increased significantly the levels of seven phenolics during ripening (e.g., 5-caffeoylquinic acid, 7.85-fold; caffeoyl-hexose I, 5.51-fold; ferulic acid, 4.09-fold), all alkaloids (e.g., dehydrolycoperoside I, 4.0-fold; dehydrolycoperoside II, 3.95-fold; hydroxy-α-tomatine II, 3.17-fold), dehydrophaseic acid hexose (2.62-fold), and azelaic acid (2.02-fold) (Tables 2 and S2).

Correlations between the levels of phenolic compounds, TP content, and antioxidant activity were only found after the ripening period. The levels of most phenolics were correlated with TP content ($r = 0.81–0.96$, $p \leq .05$), except for ferulic acid and naringenin chalcone; the highest correlation values ($p \leq .01$) were for caffeoyl-hexose I ($r = 0.96$) and chlorogenic acid ($r = 0.94$). On the other hand, DPPH activity was correlated with phenolics ($r = 0.82–0.89$, $p \leq .05$) except for *N*-feruloyl putrescine, naringenin chalcone, and rutin derivatives; chlorogenic acid showed the highest correlation ($r = 0.89$). ABTS activity also correlated with phenolics ($r = 0.83–0.93$, $p \leq .05$), except for 5-caffeoylquinic acid, ferulic acid, and *N*-feruloyl putrescine; the highest correlation was for caffeoyl-hexose I ($r = 0.93$, $p \leq .001$).

3.3 | Changes in the abundance of organic acids, sugars, and amino acids in tomato fruit in response to hot water treatment, cold storage, and ripening

GC-MS analysis identified 22 compounds that were classified into five groups: organic acids (lactic, fumaric, malic, isocitric, pyroglutamic, and γ-aminobutyric [GABA]), amino acids (isoleucine, glycine, serine, threonine, aspartic acid, phenylalanine, glutamine, and glutamine derivate), sugars (fructose, glucose, galactose, and sucrose), sugar alcohols (glycerol and myo-inositol), and others (phosphoric acid and adenosine) (Tables S2 and 3). The HWT before cold storage induced significant changes. Seven compounds increased their levels: four sugars (1.24–2.18-fold), glutamine (1.68-fold), lactic acid (1.36-fold), and glutamine derivate (1.35-fold). On the other hand, eight compounds decreased their content: four amino acids (isoleucine, glycine, threonine, and phenylalanine) (0.39–0.73-fold), pyroglutamic acid (0.58-fold), phosphoric acid (0.71–fold), fumaric acid (0.58-fold), and malic acid (0.82-fold) (Table 3).

After chilling stress (20 days at 5°C), both treatments (control and HWT) showed increased levels of isocitric acid (2.87-fold and 3.01-fold), GABA (2.04-fold and 2.34-fold), and adenosine (3.12-fold and 4.05-fold); lower levels were found for malic acid (0.62-fold and 0.57-fold), glycine (0.24-fold and 0.29-fold), and aspartic acid (0.66-fold and 0.66-fold). The content of lactic acid increased in the control and decreased in HWT fruit (1.63-fold and 0.54-fold), while the opposite was observed for sucrose (0.18-fold and 1.09-fold) (Table 3). Thus, control fruit had a significantly higher content of lactic acid, serine, and glycerol than HWT fruit, whereas HWT fruit exhibited a higher abundance of sucrose, GABA, and adenosine than control fruit (Table 3).

Analyzing the effect of cold storage plus ripening (20 + 7) with respect to day 0, 15 metabolites decreased significantly their levels in both treatments, and nine of them showed lower values in HWT than control fruit (Table 3). On the other hand, both treatments (control, HWT) increased the levels of glutamine (4.56-fold and 3.77-fold) and adenosine (3.6-fold and 2.25-fold). Analyzing the effect of ripening with respect to cold storage, most compounds decreased their levels and showed lower values for the HWT fruit. In contrast, two compounds increased their levels in both treatments (control and

TABLE 3 Changes in the levels of metabolites identified by GC-MS in the pericarp of control and HW-treated "Imperial" tomato fruit after cold storage and ripening

Metabolite	Treatment	Storage condition		
		0 days at 5°C	20 days at 5°C	20 days at 5°C + 7 days at 21°C
Organic acids				
Lactic acid	Control	1^{Bb}	1.63 ± 0.20^{Aa}	0.45 ± 0.03^{Ca}
	HW	1.36 ± 0.06^{Aa}	0.54 ± 0.01^{Bb}	0.21 ± 0.03^{Cb}
Fumaric acid	Control	1^{Aa}	1.08 ± 0.11^{Aa}	ND
	HW	0.58 ± 0.10^{Bb}	1.13 ± 0.13^{Aa}	ND
Malic acid	Control	1^{Aa}	0.62 ± 0.03^{Ba}	0.44 ± 0.05^{Ca}
	HW	0.87 ± 0.05^{Ab}	0.57 ± 0.08^{Ba}	0.25 ± 0.01^{Cb}
Isocitric acid	Control	1^{Ba}	2.87 ± 0.6^{Aa}	2.39 ± 0.48^{Aa}
	HW	1.19 ± 0.20^{Ba}	3.01 ± 0.09^{Aa}	1.44 ± 0.28^{Bb}
Amino acids				
Isoleucine	Control	1^{Aa}	0.34 ± 0.11^{Ba}	0.47 ± 0.02^{Ba}
	HW	0.39 ± 0.09^{Ab}	0.37 ± 0.01^{Aa}	0.27 ± 0.01^{Ab}
Glycine	Control	1^{Aa}	0.24 ± 0.01^{Ba}	0.33 ± 0.04^{Ba}
	HW	0.47 ± 0.09^{Ab}	0.29 ± 0.07^{Ba}	0.21 ± 0.03^{Ba}
Serine	Control	1^{Aa}	0.88 ± 0.09^{Aa}	0.52 ± 0.02^{Ba}
	HW	0.88 ± 0.18^{Aa}	0.51 ± 0.06^{Bb}	0.39 ± 0.09^{Ba}
Threonine	Control	1^{Aa}	0.40 ± 0.13^{Ba}	0.49 ± 0.02^{Ba}
	HW	0.52 ± 0.05^{Ab}	0.45 ± 0.07^{ABa}	0.31 ± 0.05^{Bb}
Aspartic acid	Control	1^{Aa}	0.66 ± 0.10^{Ba}	1.01 ± 0.09^{Aa}
	HW	0.99 ± 0.28^{Aa}	0.66 ± 0.01^{Ba}	0.98 ± 0.24^{Aa}
Phenylalanine	Control	1^{Aa}	0.64 ± 0.01^{Ca}	0.81 ± 0.02^{Ba}
	HW	0.73 ± 0.02^{ABb}	0.66 ± 0.03^{ABa}	0.58 ± 0.13^{BCb}
Glutamine	Control	1^{Ba}	1.18 ± 0.01^{Ba}	4.56 ± 0.29^{Aa}
	HW	1.68 ± 0.32^{Bb}	1.24 ± 0.13^{Ba}	3.77 ± 0.79^{Ab}
Glutamine derivate	Control	1^{Ab}	1.21 ± 0.10^{Aa}	1.20 ± 0.09^{Aa}
	HW	1.35 ± 0.09^{Aa}	1.37 ± 0.19^{Aa}	1.05 ± 0.14^{Ba}
Sugars				
Fructose	Control	1^{Ab}	1.21 ± 0.09^{Aa}	0.50 ± 0.13^{Bb}
	HW	1.27 ± 0.05^{Aa}	1.15 ± 0.00^{Aa}	0.75 ± 0.21^{Ba}
Glucose	Control	1^{Ab}	1.03 ± 0.11^{Aa}	0.49 ± 0.07^{Bb}
	HW	1.24 ± 0.09^{Aa}	1.13 ± 0.04^{Aa}	0.82 ± 0.07^{Ba}
Sucrose	Control	1^{Ab}	0.18 ± 0.03^{BCb}	0.57 ± 0.10^{ABa}
	HW	2.18 ± 0.30^{Aa}	1.09 ± 0.01^{Ba}	0.34 ± 0.08^{Ca}
Galactose	Control	1^{Cb}	1.10 ± 0.11^{Aa}	0.75 ± 0.22^{Ba}
	HW	1.50 ± 0.09^{Aa}	1.34 ± 0.37^{Aa}	0.73 ± 0.11^{Ba}
Sugar alcohols				
Glycerol	Control	1^{Ba}	1.52 ± 0.05^{Aa}	0.23 ± 0.02^{Ca}
	HW	0.90 ± 0.20^{Aa}	0.86 ± 0.07^{Ab}	0.13 ± 0.04^{Ba}
Myo-inositol	Control	1^{Aa}	1.05 ± 0.04^{Aa}	0.33 ± 0.15^{Ba}
	HW	1.04 ± 0.04^{Aa}	1.08 ± 0.02^{Aa}	0.40 ± 0.08^{Ba}
Others				
Phosphoric acid	Control	1^{Aa}	1.00 ± 0.11^{Aa}	0.48 ± 0.06^{Ba}
	HW	0.71 ± 0.07^{Bb}	1.00 ± 0.06^{Aa}	0.38 ± 0.08^{Ca}

(Continues)

TABLE 3 (Continued)

Metabolite	Treatment	Storage condition		
		0 days at 5°C	20 days at 5°C	20 days at 5°C + 7 days at 21°C
Pyroglutamic acid	Control	1^{Aa}	0.54 ± 0.03^{Ba}	0.65 ± 0.01^{Ba}
	HW	0.52 ± 0.05^{Ab}	0.41 ± 0.03^{ABa}	0.31 ± 0.06^{Bb}
GABA	Control	1^{Ba}	2.04 ± 0.24^{Aa}	0.65 ± 0.16^{Ca}
	HW	0.98 ± 0.09^{Ba}	2.34 ± 0.31^{Aa}	0.67 ± 0.03^{Ca}
Adenoside	Control	1^{Ba}	3.12 ± 0.51^{Ab}	3.60 ± 0.17^{Aa}
	HW	0.80 ± 0.01^{Ca}	4.05 ± 0.13^{Aa}	2.25 ± 0.56^{Bb}

Note: Values are relative to the level of the control fruit after 0 days at 5°C; they are the mean ± *SD* of three replicates. Different letters in the same row (A, B, and C) or column (a and b) indicate significant differences (Fisher, $p < .05$) between storage conditions and treatments, respectively.
Abbreviations: HW, hot water; ND, not detected.

HWT): aspartic acid (1.53-fold and 1.48-fold) and glutamine (3.86-fold and 3.04-fold) (Table 3). At the end of the ripening period, HWT fruit had a significantly lower content of organic acids (lactic, malic, and isocitric), amino acids (isoleucine, threonine, phenylalanine, and glutamine), and other metabolites (pyroglutamic acid and adenosine) than control fruit (Table 3). Fructose, glucose, and myoinositol were the only compounds that showed higher content in HWT than control fruit after the ripening period.

4 | DISCUSSION

4.1 | Chilling injury tolerance in hot water-treated tomatoes is associated with increased phenolics content and antioxidant activity

Considering the chilling injury index data (Table 1 and Figure S1), the HWT of tomato fruit cv Imperial induced tolerance against this disorder. These results agree with previous observations that HWT reduces the chilling injury symptoms in this tomato cultivar (Cárdenas-Torres et al., 2020; Salazar-Salas et al., 2017). In this regard, the response of plants to different stress conditions (e.g., heat, cold) implies the production of reactive species, and plant protection is mediated by the activation of their enzymatic and nonenzymatic antioxidant systems, among other mechanisms (Giordano et al., 2021; Gonzalez et al., 2019; Zhang et al., 2019). In the present study, we first evaluated the total phenolics (TP) content and the in vitro antioxidant activity to determine the participation of the nonenzymatic antioxidant system in the HWT-induced chilling tolerance in tomato fruit cv Imperial. HWT fruit with less incidence of CI symptoms showed significantly higher TP content and antioxidant activity than control fruit (Table 1). The effect of the HWT on phenolics content and antioxidant activity depends on the tomato variety and its developmental stage: HWT "BHN-602" tomatoes at the breaker turning stage and stored under nonchilling conditions (1 week at 12.5°C) showed an increase in phenolics after the fruits were ripened, but the opposite was observed when the tomatoes were stored for 1 week at 5°C (Loayza et al., 2021). HWT did not affect the phenolics content in mature green fruit at any of the evaluated conditions; furthermore, there were no significant differences in the antioxidant activities measured by the ORAC and FRAP methods (Loayza et al., 2021). Considering the results obtained with the cv. Imperial, the mechanisms involved in the HWT-induced CI tolerance were further investigated comparing the metabolic profiles of control and HW-treated fruit stored under chilling conditions.

4.2 | Changes in the secondary metabolism associated with chilling injury and the tolerance induced by hot water treatment

The composition of tomato depends on the variety and developmental stage: Naringenin chalcone, chlorogenic acid, and organic acids (*p*-coumaric, caffeic, and ferulic) are commonly found in tomato fruit, being rutin (quercetin 3-*O*-rutinoside) the main flavonoid in ripened tomatoes of most cultivars (Slimestad & Verheul, 2009). The described composition was similar to that found in tomato cv. Imperial, being naringenin chalcone and chlorogenic acids the main phenolics (Table 2).

The HWT applied before cold storage increased the synthesis of 6 out of 14 phenolics (e.g., 5-caffeoylquinic acid, coumaroylhexose, naringenin chalcone, rutin derivatives) but decreased the accumulation of chlorogenic acid (3-caffeoylquinic acid) and tricaffeoylquinic acid (Tables 2 and S2). These results correspond with previous reports showing that plants under heat stress increase their content of flavonoids (e.g., quercetin derivatives) (Giordano et al., 2021). Besides, the transcript levels of chalcone synthase and chalcone isomerase, two genes involved in the synthesis of flavonoids were induced after 2 and 24 hr in Micro-Tom fruit with HWT (40°C, 7 min), but there was a reduction in the expression of the gene encoding hydroxycinnamoyl CoA quinate transferase, an enzyme related to the synthesis of caffeoylquinic acids (Cruz-Mendívil et al., 2015). In addition, coumaroyl and malonyl are the precursors of naringenin chalcone, a metabolite

synthesized in the first step of the flavonoid biosynthetic pathway (Bovy et al., 2007). The alkaloids dehydrolycoperoside and esculeoside A1 increased with the HWT (Tables 2 and S2); these changes agreed with the accumulation of alkaloids in plants exposed to biotic and abiotic stress (Lurie & Pedreschi, 2014). There was also a decrease in tryptophan content and an increase in azelaic acid levels due to the HWT (Tables 2 and S2). Tryptophan is the precursor of melatonin and indoleacetic acid, and both share hormone characteristics. Melatonin is produced as a protective agent when plants are exposed to stress conditions, involving its antioxidant and gene regulation properties that include the activation of the phenylpropanoid pathway (Hameed et al., 2020; Jannatizadeh et al., 2019). Azelaic acid is a fatty acid-derived signaling molecule involved in plant defense mechanisms and also induces the phenylpropanoid pathway (Egorova & Tarchevsky, 2018).

After cold storage, the content of most phenolic compounds decreased but their levels were similar between the treatments (Tables 2 and S2). Red Rose tomato fruit exposed to cold (10°C) showed increased levels of total phenolics and flavonoids after seven days of storage but then the values decreased towards the end of the experiment (21 days) (Alenazi et al., 2020). On the other hand, the levels of *N*-feruloyl putrescine increased in both control and HWT fruit, registering lower values for the HWT fruit (Tables 2 and S2). Putrescine accumulates under low-temperature storage, and it was associated with acclimation to chilling stress (Cook et al., 2004), a pattern observed in a cold-tolerant potato tuber (*Solanum acaule*) (Kou et al., 2018) and tomato fruits and plants treated with melatonin (Ding et al., 2017; Jannatizadeh et al., 2019), as well as in Micro-Tom tomato fruit exposed to cold shock (Gonzalez et al., 2019). Thus, the increased levels of *N*-feruloyl putrescine in the control fruit could be part of an acclimation mechanism (Table 2). Moreover, control fruit had significantly higher levels of coumaroyl-hexose, caffeoyl-hexose I, caffeic acid, tricaffeoylquinic acid, and kaempferol-3-*O*-rutinoside than HWT fruit (Tables 2 and S2). The accumulation of caffeoylquinic acids has been associated with protection against chilling injury in peach (Lauxmann et al., 2014), but the levels of these compounds decreased in Micro-Tom tomatoes treated with hot water and stored at 2.5°C for 14 days (Luengwilai, Saltveit, et al., 2012). The alkaloids esculeoside AII and hydroxy-α-tomatine II were detected after the cold storage in both control and HWT fruit, but their levels were higher in the latter (Tables 2 and S2). This suggests a higher negative effect of the cold stress on the control fruit compared with HWT fruit. Tryptophan decreased in the control fruit but increased in the HWT fruit (Tables 2 and S2). In potato tuber, cold storage decreases the levels of tryptophan (Datir et al., 2020), as it was registered in the control fruit; thus, the increased levels of tryptophan in the HWT fruit could be important in the reduction of cold-induced damage.

The ripening process is important in the development of visible CI symptoms in susceptible fruits, which may be associated with the overproduction of reactive oxygen species (Albornoz et al., 2019). At the end of the ripening period, HWT fruit accumulated greater quantities of caffeic acid and its derivatives (caffeoyl-hexose I, chlorogenic acid, and 5-caffeoylquinic acid), ferulic acid, *N*-feruloyl putrescine, and flavonoids (kaempferol-3-*O*-rutinoside, rutin pentoside, and rutin) than control fruit (Tables 2 and S2). These results correspond with the significant correlation found between the levels of these compounds and total phenolic content and antioxidant activity (ABTS and DPPH), suggesting that they are the main compounds responsible for the increase in total phenolics and antioxidant activity in HWT fruit. The better profile of phenolics in HWT fruit supported that its ripening metabolism is less affected than that of control fruit. Tomato fruit nonexposed to cold stress shows increased levels of phenolics after ripening (Slimestad & Verheul, 2009). Flavonoids have antioxidant properties, and HWT fruit showed higher flavonoid accumulation than control fruit, reducing the oxidative stress during the ripening process and alleviating the CI symptoms. The importance of flavonoids in the acquisition of freezing tolerance was demonstrated in *Arabidopsis thaliana*, where plants with mutations in flavonoid biosynthetic genes showed a strong susceptibility to freezing temperatures (Schulz et al., 2016). Moreover, Aghdam et al. (2019) demonstrated that tomato fruit cv. Izmir pre-treated with phenylalanine were cold tolerant, showing higher levels of phenolics and flavonoids than the untreated fruit. Furthermore, genes of secondary metabolism and flavonoid biosynthesis were upregulated in tomato fruit cv. Micro-Tom exposed to cold stress (4°C/28 days) (Zhang et al., 2019).

Dehydrolycoperoside and esculeoside are alkaloids whose content increases during the ripening of tomato fruit (Katsumata et al., 2011; Moco et al., 2007). HWT ripened fruit showed higher levels of all detected alkaloids than control ripened fruit but hydroxy-α-tomatine (Tables 2 and S2). Hydroxy-α-tomatine is produced during the conversion of α-tomatine (bitter flavor) to the nonbitter compounds dehydrolycoperoside and esculeoside (Ballester et al., 2016). The higher accumulation of hydroxy-α-tomatine in ripe control than HWT fruit corresponds with the lower content observed in the control for dehydrolycoperoside and esculeoside, suggesting a more uniform ripening (Tables 2 and S2).

Azelaic acid has been used as a marker of nonenzymatic lipid oxidation in plants (Zoeller et al., 2012) and as a signal of the systemic acquired resistance activation associated with salicylic acid accumulation (Jung et al., 2009). The accumulation of azelaic acid after cold storage was significantly higher in control than HWT fruit (Tables 2 and Table S2), and it could be an indicator of the damage caused by the oxidative stress due to chilling conditions, whereas the result observed after the ripening period could be associated with the low incidence of decay observed in HWT fruit (Figure S1). The increase in dehydrophaseic acid hexose observed in HWT fruit after ripening (Tables 2 and S2) has been previously reported by Moco et al. (2007) in red tomato ripened under normal conditions. Dehydrophaseic acid-hexose is involved in abscisic acid biosynthesis, a phytohormone whose exogenous application in mature green cherry tomatoes enhanced the enzymatic and nonenzymatic antioxidant activity and gene expression related to the phenylpropanoid pathway (Tao et al., 2020).

4.3 | Adjustments in the tomato primary metabolism associated with chilling injury development and the tolerance induced by hot water treatment application

The HWT increased the levels of all detected tomato sugars, but the greatest effect was sucrose (2.18-fold) (Table 3). This pattern suggested changes in metabolism and agreed with the accumulation of glucose, sucrose, and fructose in leaves and roots of heat-treated plants (e.g., lettuce, chicory, chilli) (Giordano et al., 2021). Heat-treated fruit (e.g., citrus, peach, potato, tomato) also showed an increased accumulation of sugars (Lurie & Pedreschi, 2014). The highest level of sucrose agreed with the results of Iwahashi and Hosoda (2000) who reported a lower accumulation of invertase protein in tomato fruit after the application of thermal treatment. Furthermore, sucrose availability has been related to the heat stress tolerance of tomato fruit (Almeida et al., 2020; Alsamir et al., 2021).

The decrease in the content of four tomato amino acids by the HWT (Table 3) could be due to changes in carbohydrate metabolism. During postharvest, sugars are the main source of energy, and the increased levels of these compounds in HWT fruit must be related to lower rates of glycolysis and Krebs cycle, resulting in lower levels of some amino acids in tomato. Moreover, the increased content of glutamine derivatives by the HWT (Table 3) could be due to the participation of these compounds in the stress metabolic adjustment of tomatoes. Heat-treated mandarins (Yun et al., 2013) and peaches (Lauxmann et al., 2014) showed a similar metabolite pattern and seem to be a common response to heat treatment. In this regard, transcriptomic analysis of tomato fruit (cv. Alisa Craig) showed that heat treatment induced the downregulation of carbohydrate and Krebs cycle metabolic genes (Almeida et al., 2020). Our data and published information support an important role of glutamine for the cold tolerance induced by HWT in tomato cv. Imperial. The reduction in the abundance of pyroglutamic acid in HWT compared with control fruit was accompanied by an increase of glutamine and glutamine derivate (Table 3). Pyroglutamic acid can be converted to glutamic acid by 5-oxoprolinase in plants (Mazelis & Creveling, 1978) and fruits (Du et al., 2016), which is a precursor of glutamine. Du et al. (2016) observed a higher accumulation of 5-oxoprolinase in the peel of heat-treated banana fruit compared with untreated fruit. Glutamine is an indirect precursor of polyamines through arginine metabolism, which is activated by heat stress and has been associated with chilling tolerance in tomato fruit (Zhang et al., 2013). On the other hand, the oxidation of sugars provides energy to the cell, so changes in carbohydrate concentration suggest variations in cell energy demands. In this sense, the heat stress increased the levels of lactic acid (1.36-fold) and decreased the content of fumaric (0.58-fold) and malic acids (0.87-fold) (Table 3), suggesting that the HWT partially induced the anaerobic metabolism. In this respect, Romieu et al. (1992) reported that fruits subjected to heat stress during maturation showed partial anaerobic metabolism.

After cold storage, control fruit showed increased levels of lactic acid, isocitric acid, galactose, glycerol, GABA, and adenosine, whereas isoleucine, glycine, threonine, aspartic acid, phenylalanine, sucrose, and pyroglutamic acid decreased their levels. Moreover, the HWT fruit showed higher levels of isocitric acid, sucrose, GABA, and adenosine than the control fruit (Table 3). The significantly higher accumulation of sucrose in HWT fruit than the control fruit (Table 3) may be part of an acclimation mechanism to stabilize the membrane structure, avoiding leakage and membrane protein inactivation due to the dehydration caused by cold stress (van den Bogaart et al., 2007). After ripening, control fruit was more damaged than HWT fruit. A metabolic adjustment of control fruit to cold storage was the activation of fermentation metabolism. Recently, Gonzalez et al. (2019) reported the accumulation of ethanol and the upregulation of genes involved in the fermentation process in tomato fruit exposed to chilling stress. In this sense, Cruz-Mendivil et al. (2015) reported a higher respiration rate in Micro-Tom tomato fruit with chilling injury symptoms compared with HWT tomato fruit, whereas Yang et al. (2009) reported that the structure and function of mitochondria in cherry tomato fruit were negatively affected by cold stress and that HWT reduced the damage in this organelle. Therefore, the increased accumulation of lactic acid content in control fruit could be due to the activation of the anaerobic metabolism, as a consequence of mitochondrial damage, to supply the required energy for maintaining cellular homeostasis. By contrast, HWT may have protected the cells from mitochondrial damage by increasing the accumulation of heat shock proteins and chaperonins as was previously reported by Salazar-Salas et al. (2017). On the other hand, most amino acids decreased their levels after cold storage in control and HWT fruit but glutamine and glutamine derivatives (Table 3). The accumulation of amino acids has been associated with cold tolerance, but the results vary among species and cultivars of the same species. Our results contrast with those reported by Kou et al. (2018) for *Solanum acaule* tuber, a potato cold-resistant material stored at 4°C. They observed an increase in the levels of tyrosine, isoleucine, and valine, whereas those of glutamic acid decreased, suggesting that higher levels of amino acids are contributing to cold tolerance. However, the amino acids associated with cold tolerance vary among different potato cultivars (Datir et al., 2020). Carbon dioxide-treated tomato fruit improved its cold tolerance, such tolerance was associated with increased levels of alanine, valine, lysine, glutamic acid, serine, glycine, and tyrosine after cold storage at 4°C for 14 days (Park et al., 2021). Glutamate, glutamine, aspartate, and asparagine increased in mature green Micro-Tom tomato fruit after cold storage at 4°C for 28 days (Gonzalez et al., 2019), whereas another report with Micro-Tom fruit showed that alanine and leucine increased their levels after cold storage (4°C/28 days) (W. F. Zhang et al., 2019).

Considering these data, glutamine seems to be a key factor in the cold tolerance induction of tomato cv. Imperial, since it increased with the HWT ($p < .05$), and after cold storage, the values for the HWT fruit were still slightly higher than those of control fruit ($p > .05$) (Table 3). Both control and HWT fruit increased their levels of GABA and adenosine after cold storage with higher values for HWT fruit (Table 3). GABA is produced in response to biotic and abiotic stresses and is synthesized from glutamine. Then, GABA is transported from cytosol to mitochondria where is converted to succinate, which provides electrons to the Krebs cycle and improves the energetic status of the cells (Morteza Soleimani Aghdam et al., 2018). Potato tubers of five cultivars were exposed to cold storage at 4°C, one of them showed higher levels of GABA, and the five cultivars increased their levels of adenosine (Datir et al., 2020). Another study showed the GABA levels were also increased in cold-stored potato tubers of two cultivars (*S. acaule* and *S. tuberosum*) (Kou et al., 2018). GABA is proposed as a key metabolite in the induction of cold tolerance by melatonin treatment of tomato fruit since tomato shows the high-energy status (Jannatizadeh et al., 2019). However, Micro-Tom tomato stored at 4°C for 28 days decreased its levels of GABA (Gonzalez et al., 2019).

The ripening process decreased the content of organic acids (lactic, fumaric, malic, and isocitric) in both control and HWT fruit, but the abundance of these compounds was significantly higher in control fruit with more severe CI symptoms (Table 3). Previously, we observed an increase in the accumulation of triose phosphate isomerase and enolase in HWT tomato after storage for 20 days at 5°C and an increase in cytochrome *c* reductase-processing peptidase after the ripening period (20 days at 5°C + 7 days at 21°C) (Salazar-Salas et al., 2017). Compared with the content of organic acids in ripened tomato cv. Imperial (Table 3), the levels of most organic acids of Micro-Tom tomato fruit decreased (Gonzalez et al., 2019) as well as those of tomato cv. Defunis with improved cold tolerance by carbon dioxide treatment (Park et al., 2021). Among the amino acids, only the levels of glutamine increased in tomato, and the HWT fruit showed lower levels of all amino acids (Table 3). Compared with the levels of amino acids in ripened tomato cv. Imperial, the levels in ripened Micro-Tom tomato increased (Gonzalez et al., 2019). Free amino acids content in ripened cv. Micro-Tom tomato fruit has been mainly associated with proteolysis (Sorrequieta et al., 2010). Thus, the significantly higher levels of free amino acids in control fruit (Table 3) could be related to the degradation of damaged and unfolded proteins. In this sense, Re et al. (2012) associated a decrease in the content of insoluble-membrane proteins of ripe pre-chilled tomato fruit with higher autoproteolytic activity. The reduction in sugar levels in ripened tomatoes (Table 3) showed the same pattern found in ripened Micro-Tom tomatoes (Gonzalez et al., 2019). Fructose and glucose levels were higher in ripened HWT fruit (Table 3), and they may be osmolytes involved in chilling tolerance induced by hot water treatment. These metabolites are produced from sucrose hydrolysis during tomato ripening, a pathway irreversibly affected by low temperature in the pericarp of tomato fruit (Albornoz et al., 2019). Besides, they are key compounds for energy production and synthesis of antioxidants and reducing compounds like ascorbic acid (Linster & Clarke, 2008) and NADPH since they can feed the oxidative pentose phosphate pathway (Wang et al., 2013). A higher antioxidant activity could decrease the oxidative stress produced during ripening and cold storage. Cold stress modifies the structure and chemical composition of cell membranes decreasing their fluidity and permeability (Lyons, 1973).

All sugar alcohols and other compounds decreased their levels in ripened tomatoes, and glycerol was in higher abundance in control fruit after 20 days at 5°C (Table 3). Glycerol is a component of membrane lipids, and the higher level could be the result of membrane damage, a phenomenon evidenced in previous research where untreated fruit showed higher electrolyte leakage and malondialdehyde content than HWT fruit (Salazar-Salas et al., 2017). Leisso et al. (2015) reported that chilling injury development in apple was accompanied by a sharp increase in glycerol, which was associated with triacylglycerides catabolism.

5 | CONCLUSIONS

The activation of the stress response and induction of the CI tolerance in tomato cv. Imperial could be started by the increased levels of azelaic acid, glutamine, and tryptophan in HWT fruit, which in turn increased the biosynthesis of phenolics (e.g., 5-caffeoylquinic acid), sugars, and alkaloids. Besides, *N*-feruloyl putrescine, esculeoside AII, and hydroxy-α-tomatine II were produced in response to cold stress, registering lower levels in HWT fruit than control fruit. Furthermore, the better metabolic performance of the HWT fruit under cold storage was corroborated by a better aerobic metabolism in ripened fruit and higher accumulation of most metabolites, highlighting the content of 5-caffeoylquinic acid, dehydrolycoperoside, hydroxy-α-tomatine II, and escualoside.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Francisco Delgado-Vargas: Conceptualization; Writing—review & editing. **Milton Vega-Alvarez:** Methodology. **Alexis Landeros-Sánchez:** Investigation. **Gabriela López-Angulo:** Methodology. **Nancy Yareli Salazar-Salas:** Methodology. **Maria Fernanda Quintero-Soto:** Methodology. **Karen Virginia Pineda-Hidalgo:** Methodology. **José**

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