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ORIGINAL PAPER

Chilling injury tolerance induced by quarantine hot water treatment in mango fruit is associated with an increase in the synthesis of gallotannins in the pulp

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Abstract

The quarantine hot water treatment (HWT) reduces the chilling injury (CI) symptoms in mango fruit. In 'Keitt' mango this effect is associated with up-regulation of the secondary metabolism in the peel, specifically higher accumulation of gallic acid derivatives, but the metabolic changes of the pulp are unknown. This study analyzed metabolic and expression changes in the pulp of 'Keitt' mango to further elucidate the mechanisms associated with the HWT-induced CI tolerance. Mangoes with HWT (46.1 °C, 90 min) and control were stored under CI conditions (20 days at 5 °C) and then ripened (7 days at 21 °C). Methanol extracts were analyzed for total phenolics (TP), antioxidant capacity (ABTS, DPPH, FRAP) and phenolic profiles by UPLC-DAD-MS; the expression of genes encoding phenylalanine ammonium lyase (PAL), chalcone synthase (CHS) and glucosyltransferase (UGT) was analyzed by RT-qPCR. HWT alleviated CI symptoms and protected the membrane integrity as evidenced by lower CI index, electrolyte leakage, and malondialdehyde content values. The metabolites identified (20) were classified as gallotannins, gallic acid derivatives, benzoic acid derivatives, phaseic acid derivatives, and flavonoids. After cold storage and ripening, HWT fruit had higher levels of gallotannins, the most abundant compounds, and other metabolites such as *p-h*ydroxybenzoic acid hexoside and dihydrophaseic acid hexoside. HWT fruit also had higher expression of the *UGT* gene and higher values of TP and antioxidant capacity (ABTS, DPPH, and FRAP). Thus, the HWT-induced CI tolerance in mango fruit is associated with an enhanced antioxidant capacity in the pulp due to the increased synthesis of gallotannins.

Keywords Mango pulp · Chilling injury · Hot water treatment · Metabolomics · Gallotannins

Introduction

Mango (*Mangifera indica* L.) is one of the most important fruit crops worldwide; Mexico was the fourth producer and the first exporter of mango in 2020 [1, 2]. The cultivar Keitt is highly consumed due to its aroma and flavor [3], but it is highly susceptible to chilling injury (CI), a physiological disorder that occurs when the fruit is stored below 13°C and affects its quality due to the development of symptoms such as poor aroma, lenticel darkening, uneven color development, pitting, and decay [4–8]. Chilling stress results in metabolic imbalance due to excess production of reactive oxygen species (ROS) [9], membrane stiffness and permeability [7, 10], and deficiency in energy production [11]. In this regard, low temperature storage activates the catabolism of fatty acids, favoring the accumulation of saturated over unsaturated fatty acids, membrane damage, and lipid peroxidation [7]. Cold stress also affects negatively the accumulation of phenolic compounds and ascorbic acid [12, 13], and volatile metabolites related to fruit aroma [8, 14].

Mango destined to the export market receives a quarantine hot water treatment (HWT) for pest control [15], which also reduces the CI symptoms in the cultivar Keitt

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[4]. In the pulp, this effect has been associated with a reduction in electrolyte leakage and malondialdehyde (MDA) content, an increase in the activity of antioxidant and cell wall enzymes and higher accumulation of total phenolics and carotenoids with respect to the non-treated fruit [4, 16]. Omics studies of the peel from 'Keitt' mango with HWT and non-treated revealed that the CI tolerance induced by the heat treatment was associated with greater accumulation of heat shock proteins, antioxidant and cell wall enzymes, pathogenesis-related proteins, and enzymes involved in the secondary and energy metabolism [6]. This tolerance was also associated with an increase in the ratio of unsaturated/saturated fatty acids and higher accumulation of simple sugars and gallic acid derivatives, specifically gallotannins and galloylquinic acids, which are the most abundant compounds in the peel [5]. Nevertheless, the metabolic responses of mango fruit to HWT depend on several variables such as the physical and chemical composition of the different tissues. In this sense, the changes in the metabolome and gene expression associated with CI tolerance in the pulp of mango fruit are unknown. The aim of this research was to analyze changes in phenolic profiles and the expression of genes related to the secondary metabolism in the pulp of 'Keitt' mango fruit in response to HWT, cold storage and ripening to gain further insight into the mechanisms of the CI tolerance induced by HWT.

Materials and methods

Plant material and postharvest treatments

Mango fruit (*Mangifera indica* L.) of the cultivar Keitt were obtained from an orchard located in Culiacán, Sinaloa, México (24° 47′ 40″ N, 107° 30′ 59″ W). Mature green fruit were harvested based on firmness (119 ± 8 N) [17] and selected based on weight (500–600 g), color uniformity, and absence of damage. After washing the mangoes with sodium hypochlorite (200 mg L^{-1}), two groups of 45 fruit each were formed; one received the quarantine HWT indicated by USDA-APHIS [15] for mango export (46.1 °C, 90 min) and the other was used as control. Control and HWT fruit were air-dried at 21 °C, stored at 5 °C for 20 days and then at 21 °C for 7 days (80–90% relative humidity) for ripening and visualization of CI symptoms. Mango pulp samples from both treatments (three replicates of five fruit each) were obtained before cold storage (0 days at 5 °C), after chilling stress (20 days at 5 °C), and after ripening (20 days at 5 °C plus 7 days at 21 °C). One half of the pulp sample from each replicate was frozen and the other was lyophilized. Both samples were stored at − 70 °C until use.

Reagents and solvents

The reagents purchased from Sigma-Aldrich (St. Louis, MO, USA) were standards (purity > 99%) of gallic acid, abscisic acid, hydroxybenzoic acid, quercetin, rutin, myricetin and trolox, the radicals (purity > 98%) 2,2′-azinobis(3-ethylbenzothiazolin)-6-sulfonic acid (ABTS), 2,2-diphenyl-1-pycrilhydrazyl (DPPH) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), analytical grade (> 98%) mannitol, formic acid, NaCl, and molecular biology grade (purity > 99%) cetyltrimethylammonium bromide (CTAB), tris base, polyvinylpyrrolidone (PVP) K40, β-mercaptoethanol, EDTA, SDS and diethyl pyrocarbonate (DEPC). The analytical grade solvents ethanol, isoamyl alcohol, chloroform, hydrochloric acid, hexane and ethyl acetate were obtained from J.T. Baker (Phillipsburg, NJ, USA). LC/MS grade water, methanol and acetonitrile were from Thermo Fisher Scientific (Waltham, MA, USA).

Chilling injury index (CII)

Chilling injury index (CII) was determined according to López-López et al. [4]. The CI symptoms evaluated were uneven color development (U), pitting (P), and decay (D). The CI symptoms assessment was visually performed in fifteen fruit per treatment/storage condition (five per replicate). The injury level (IL) for each symptom was estimated using a five-point hedonic scale based on the percentage of damaged surface tissue: 0 = no tissue injury, 1 = 1% to 25% of tissue injury, 2 = 26% to 50% of tissue injury, 3 = 51% to 75% tissue injury and 4 = > 76% of tissue injury. The CII was estimated using the following formula: CII = (ILU + ILP + ILD)/3.

Physiological and pulp color parameters

For electrolyte leakage (EL), pulp cylinders of 7 mm diameter (six per replicate) were washed with deionized water, covered with 25 mL of a 0.1 mol/L mannitol solution and incubated for 2 h at 25 °C under constant shaking. The conductivity of the solution was measured (Hanna Instruments EC/TDS, CDMX, MEX) after incubation and again after autoclaving (121 °C, 10 min) and cooling at 25 °C to release all the electrolytes. EL was expressed as percentage [4]. Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) technique [18] and expressed as nmol/g on a fresh weight basis. Pulp color parameters (a* and b*) were evaluated using a colorimeter CR-200 (Minolta, Ramsey, NJ, USA) and one measurement was taken in the center of each cut cheek.

Preparation of methanol extracts

Freeze-dried pulp samples from each replicate (five fruit) were mixed and ground. About 1 g of sample was mixed with 15 mL of methanol, sonicated for 15 min at 25 °C, agitated for 15 min with an orbital shaker, and centrifuged for 15 min at 12,000×*g* and 25 °C (5804R, Eppendorf, Hamburg, Germany). The supernatant was collected and the extraction was repeated two times; the recovered supernatant (45 mL) was adjusted to pH 2 with HCl and then washed three times with 15 mL of hexane. The methanol extract obtained was dried (Büchi Waterbath B-48, Brinkmann Instruments, FL, USA) and stored at − 70 °C until use for the analysis of phenolics and antioxidant capacity. For the analysis of flavonoids, the methanol extract was extracted several times with ethyl acetate until the color disappeared; the recovered ethyl acetate extract was dried and stored at − 70 °C until use. Before the analysis of metabolites, both extracts were resuspended in 1 mL of methanol, passed through a syringe filter (PVDF, 0.45 µM, Pall, FL, USA), and a C18 cartridge (C18 ec chromafix®, Macherey–Nagel, Allentown, PA, USA).

Analysis of metabolites by UPLC-DAD-MS

UPLC-DAD-MS analysis was performed according to Vega-Alvarez et al. [5] with some modifications. The methanol (3 µL) and the ethyl acetate (10 µL) extracts were used to analyze compounds detected at 280 and 350 nm, respectively. They were injected into an ACCELA UPLC-DAD system (Thermo Scientific, CA, USA) and separated with an ACE Excel C18 column (3 µm, 150 × 3 mm) (Advanced Chromatography Technologies Ltd., Aberdeen, UK). The mobile phases used were 1% formic acid in water (A) and acetonitrile (B) running at 0.25 mL/min with the following gradient: 1% B for 4 min, 1–8% B for 3 min, 8–16% B for 2 min, 16% B for 7 min,16–23% B for 2 min, 23% B for 5 min, 23–30% B for 4 min, 30–40% B for 3 min, 40–60% B for 7 min, 60–0.5% B for 5 min. Rutin and myricetin were used as internal standards. The metabolites were identified using UV-spectra and mass spectrometry (MS) data from commercial standards or data available in the literature. The quantification was based on calibration curves of gallic acid, abscisic acid, hydroxybenzoic acid, quercetin, and myricetin (Sigma Aldrich) and the results were expressed as mg/kg on a fresh weight (FW) basis.

The UPLC-DAD was connected to a linear ion trap mass spectrometer (LTQ-XL, Thermo Scientific) with an electrospray source (ESI) to characterize the different compounds in the mixture in a single analysis based on their molecular weight and fragment information. The MS operated in both positive (ESI+) and negative (ESI−) ionization mode using the following experimental parameters: spray voltage 5 kV, capillary voltage 35 V, capillary temperature 300 °C, sheath and auxiliary gas flow rate of 25 and 15 (arbitrary units), respectively. Full scan spectra were acquired over the *m/z* range of 110 to 2000 and analyzed with the Xcalibur 2.2 software (Thermo Scientific, USA). The fragmentation for MS^n experiments was performed by collision-induced dissociation applying 10–40 V. Nitrogen and helium gases were used for drying and collision, respectively. Direct insertion of the sample was also performed to assist in the identification of the compounds.

Total phenolics content and antioxidant capacity

Total phenolics was determined in the methanol extracts using the Folin-Ciocalteu assay [19]. The results were expressed as milligrams of Gallic Acid Equivalents (GAE)/100 g FW. The antioxidant capacity (AC) was evaluated using three methods: ABTS [20], DPPH [21], and FRAP [22]. The results were expressed as µmol of Trolox Equivalents (TE)/100 g FW.

RNA isolation

Total RNA was isolated using the CTAB method [23]. Frozen mango pulp from each replicate (five fruit) was ground in the presence of liquid nitrogen. About 3 g of pulverized tissue were mixed with 10 mL of extraction buffer [20 g/L CTAB, 20 g/L PVP K40, 0.1 mol/L Tris–HCl (pH 8), 0.025 mol/L EDTA, 2 mol/L NaCl, 20 mL/L β-mercaptoethanol] and incubated for 15 min at 65 °C. The mixture was extracted two times with 10 mL of chloroform: isoamyl alcohol (24:1), centrifuged (10,000×*g*/10 min/25 °C) (5804R, Eppendorf) and the supernatant was mixed with 2.5 mL of LiCl (10 mol/L) to precipitate the RNA overnight at -20 °C. After centrifugation (10,000×*g*/10 min/5 °C), the RNA pellet was mixed with 700 µL SSTE buffer [0.1 mol/L Tris–HCl (pH 8), 1 mol/L NaCl, 5 g/L SDS, 0.001 mol/L] pre-warmed at 60 °C, the mixture was extracted three times with 700 µL of chloroform:isoamyl alcohol (24:1) and the upper phase was collected (10,000×*g*/10 min/5 °C). The RNA was precipitated by adding two volumes of pure ethanol and incubating for 2 h at − 70 °C. The pellet was washed with ethanol (0.7 L/L), dried, and resuspended in 50 µL of DEPC-treated water. The RNA was treated with DNase (Sigma-Aldrich) to remove contaminant DNA.

Gene expression analysis

Gene expression was determined by RT-qPCR. RNA was quantified spectrophotometrically and 10 ng were used for the analysis. The experiment was performed using a real time system model StepOnePlus™ (Applied Biosystems, Carlsbad, USA) and the kit SCRIPT One-Step RT-PCR

(Jena Bioscience, CA, USA). PCR primers for phenylalanine ammonia lyase (*PAL*, F = CCGTGCTGCAACCAAAATGA; R = GCAATGGCAAGACGTGTGTT), chalcone synthase (*CHS*, F = CATCTGCGTGAAGTGGGTCT; R = TTCAGT CCGAGTTTGGCCTC), UDP-glucose:galloyl-1-*O*-β-D-glucosyltransferase (*UGT*, F = AACACACTGCGGATG GAACT; R = CATTCGGATCCCGGTCTTG) and actin (*ACT*, F = TCCCAGTATTGTGGGTAGGC; R = GGC AACTCGAAGCTCATTGT) were designed using Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast); the amplicon size was specified between 100 and 250 bp with an alignment temperature of 60 °C. The amplification conditions were: 50 °C for 15 min for cDNA synthesis, then 95 °C for 5 min (denaturation), followed by 40 cycles at 95 °C (15 s) and 60 °C (1 min) with a ramp rate of 2.2 °C/s. The *Actin* gene was used as reference and the expression values were calculated relative to the non-treated fruit using the $2^{-\Delta\Delta Ct}$ method.

Data analysis

Data are the mean values of three replicates of five fruit each. Two-way ANOVA was performed and the Fisher test ($P < 0.05$) was used for mean comparisons between the treatments and between the storage conditions using STATGRAPHIC plus version 5.1 (Statistical Graphics Corporation™, MD, USA). Principal component analysis was performed to investigate the grouping and relationship of chilling injury index, physiological and pulp color parameters, metabolites, and antioxidant capacity with the different treatments using the open-source R studio program (Version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Chilling injury index (CII), physiological and quality parameters

Control fruit had significantly higher CII values and more severe CI symptoms than HWT-treated fruit after cold storage and subsequent ripening (Table 1, Online Resource 1A). Pitting and uneven color development appeared in both treatments while decay was only observed in control fruit (Online Resource 1A). The ripening process (7 days at 21 °C) increased the severity of these symptoms but the CII was always higher in control than HWT-treated fruit (Table 1). The development of CI symptoms was accompanied by an increase in electrolyte leakage (EL) and malondialdehyde content (MDA) whose values were higher in control than HWT fruit after chilling stress and ripening (Table 1).

Fruit firmness was not affected by the HWT and decreased significantly after cold storage and subsequent ripening (Table 1). Regarding the pulp color, the a* parameter increased during storage in both treatments, while b*

Table 1 Chilling injury index, physiological and quality parameters of control and HW-treated 'Keitt' mango fruit stored under chilling conditions and ripened

Parameter	Treatment	Storage condition		
		0 days at 5 °C	20 days at 5 °C	20 days at 5 °C + 7 days at 21 °C
CII	Control	ND	0.80 ± 0.10^{Ba}	1.60 ± 0.17^{Aa}
	HWT	ND	0.23 ± 0.16^{Bb}	0.80 ± 0.13^{Ab}
Electrolyte leakage (%)	Control	27.44 ± 1.21^{Ca}	43.27 ± 0.41^{Ba}	58.88 ± 2.24^{Aa}
	HWT	29.06 ± 0.37^{Ca}	36.47 ± 2.80^{Bb}	51.66 ± 1.73^{Ab}
MDA content (nmol/g)	Control	11.85 ± 1.41^{Ca}	31.15 ± 3.47^{Ba}	41.48 ± 2.83^{Aa}
	HWT	11.25 ± 1.73^{Ca}	20.17 ± 0.51^{Bb}	29.96 ± 2.03^{Ab}
Firmness (N)	Control	120.84 ± 3.84^{Aa}	79.62 ± 6.14^{Ba}	9.20 ± 0.18^{Ca}
	HWT	118.51 ± 4.00^{Aa}	75.29 ± 5.12^{Ba}	9.11 ± 0.77^{Ca}
Pulp color (a*)	Control	2.86 ± 0.01^{Ba}	3.72 ± 0.49^{Aa}	4.28 ± 0.10^{Aa}
	HWT	2.69 ± 0.30^{Ca}	4.02 ± 0.29^{Ba}	4.90 ± 0.26^{Aa}
Pulp color (b*)	Control	59.33 ± 1.05^{Aa}	60.79 ± 0.30^{Aa}	62.20 ± 0.11^{Ab}
	HWT	59.41 ± 0.77^{Ba}	60.61 ± 0.67^{Ba}	66.09 ± 1.86^{Aa}

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

CII Chilling injury index; *MDA* Malondialdehyde; *HWT* Hot water treatment; *ND* Not determined

changed only after ripening (Table 1). The b* parameter was higher in HWT than control fruit after ripening, indicating a more yellowish color (Online Resource 1B).

Effect of HWT, chilling stress and ripening on the content of metabolites in the pulp of mango fruit

Twenty metabolites were separated by UPLC-DAD in the methanol and ethyl acetate extracts (Online Resource 2). The main metabolites identified were gallic acid derivatives (*O*-galloylglucose, tetra-*O*-galloylglucose, penta-*O*-galloylglucose, hexa-*O*-galloylglucose, *O*-methyl-di-*O*-galloylglucose and galloyl di-hexoside), flavonoids (quercetin and myricetin derivatives), *p*-hydroxybenzoic acid hexoside and phaseic acid derivatives (dihydrophaseic acid hexoside isomers I, II and III) (Table 2). Galloyl glucosides showed a typical fragmentation pattern characterized by the loss of the galloyl moiety ($m/z = 152$), gallic acid ($m/z = 170$) and the presence of the pyrogallol group ($m/z = 125$). MS spectra of *O*-galloylglucose showed the molecular ion $[M\text{-}H]^-$ $m/z = 331$ and fragments of $m/z = 271$, 211, 169 (gallic acid ion) and 125 (pyrogallol group) (Table 2, Online Resource 3A) [24]. Tetra-*O*-galloylglucose exhibited the molecular ion $[M\text{-}H]^-$ $m/z = 787$ and fragments of $m/z = 635$ ($[M\text{-}H\text{-}152]^-$, loss of galloyl moiety), 617 ($[M\text{-}H\text{-}170]^-$, loss of gallic acid) and 465 ($[M\text{-}H\text{-}152\text{–}170]^-$ or $[M\text{-}H\text{-}170\text{–}152]^-$, sequential loss of the galloyl moiety and gallic acid or vice versa) (Table 3, Online Resource 3B) [25]. Quercetin derivatives were characterized for the presence of the molecular ion of the aglycone $[M\text{-}H]^-$ $m/z = 301$ and the typical fragmentation ions of $m/z = 179$ and 151 (Table 2, Online Resource 3C) [26].

The most abundant metabolites were gallic acid derivatives, specifically *O*-galloylglucose (Table 3). Before cold storage (0 days at 5 °C), HWT decreased significantly the accumulation of *O*-galloylglucose, *p*-hydroxybenzoic acid hexoside, dihydrophaseic acid hexoside, and myricetin hexoside, but it also increased the abundance of penta-*O*-galloylglucose, hexa-*O*-galloylglucose, *O*-methyl-di-*O*-galloylglucose and one gallic acid derivative (compound 3) (Table 3).

The chilling stress (20 days at 5 °C) modified the abundance of the metabolites in both treatments. In control fruit, the levels of *O*-galloylglucose and *p*-hydroxybenzoic acid hexoside decreased significantly while those of gallic acid derivatives [*O*-methyl-di-*O*-galloylglucose, tetra-*O*-galloylglucose, penta-*O*-galloylglucose, hexa-*O*-galloylglucose and gallic acid derivative (compound 3)] increased (Table 3). In HW-treated fruit, cold stress increased the abundance of *O*-galloylglucose, galloyl di-hexoside, *p*-hydroxybenzoic acid hexoside, dihydrophaseic acid hexoside, and myricetin hexoside, but it decreased the content of gallic acid derivatives (penta-*O*-galloylglucose and hexa-*O*-galloylglucose). At the end of cold storage (20 days at 5 °C), HWT fruit had significantly higher levels of *O*-galloylglucose, galloyl di-hexoside, and *p*-hydroxybenzoic acid hexoside than control samples, but the opposite was observed for tetra-*O*-galloylglucose, penta-*O*-galloylglucose, and hexa-*O*-galloylglucose.

After ripening (20 days at 5 °C + 7 days at 21 °C), the content of *O*-galloylglucose increased significantly in both treatments, but its level was significantly higher in HWT than control fruit (Table 3). Tetra-*O*-galloylglucose, penta-*O*-galloylglucose, hexa-*O*-galloylglucose and compound 3 are other gallic acid derivatives whose abundance also increased during ripening in HWT fruit reaching values significantly higher than those of the control. In addition, the abundance of *O*-methyl-di-*O*-galloylglucose, tetra-*O*-galloylglucose, gallic acid derivative (compound 3), and dihydrophaseic acid hexoside decreased during ripening in control fruit, and their levels were significantly lower than those of HWT fruit.

Total phenolic content and antioxidant capacity of mango pulp

TP content was negatively affected by chilling stress only in control fruit with a reduction of 34% (Table 4). After ripening, there was an increase in TP content only in HWT fruit reaching a value significantly higher than that of the control fruit. The AC by the three methods (ABTS, DPPH and FRAP) was affected by the HWT before cold storage (Table 4), which corresponded with the reduction observed in the levels of *O*-galloylglucose, the most abundant phenolic compound (Table 3). The chilling stress (20 days at 5 °C) reduced significantly the AC in the control fruit reaching values similar to those of the HWT fruit (Table 4). After ripening (20 days at 5 °C + 7 days at 21 °C), the HWT fruit showed a significant increase in the AC by the three methods with values significantly higher than those of the control fruit (Table 4).

Changes in expression of genes encoding enzymes involved in the synthesis of the main metabolites identified in mango pulp

The relative expression of genes encoding key enzymes involved in the synthesis of gallotannins, phenylpropanoids, and flavonoids is shown in Fig. 1. There were no significant differences in the expression of *PAL* and *CHS* between control and HWT fruit in the different storage conditions. However, the transcript levels of *UGT,* the gene encoding a glucosyltransferase that synthesizes *O*-galloylglucose, were higher in both chilled and ripened HWT fruit compared to the control fruit (Fig. 1B and C).

Table 2 Metabolites identified by UPLC-DAD-MS in the pulp of control and HW-treated mango fruit cv. Keitt after cold storage (20 days at 5 °C) and ripening (20 days at 5 °C plus 7 days at 21 °C)

Peak no	Rt	UV ƛmax	$[M-H]^-$ m/z	Tentative identification	Formula	Molecular mass	HPLC-ESI (-)-MS^n experiment m/z (% base peak)	References
Gallotannins								
1	7.27	278	331.07	*O*-galloylglucose I	$C_{13}H_{16}O_{10}$	332.18	331.07 (100); 271.05 (6.37), 211.04 (3.17), 169.22 (6.68), 125.1 (4.25)	[5, 24, 40–42]
2	8.57	278	331.09	*O*-galloylglucose II	$C_{13}H_{16}O_{10}$	332.90	331.09 (100); 271.51 (4.29), 210.90 (2.8), 210.90 (2.8), 169.07 (6.09), 125.24 (4.2)	[5, 24, 40–42]
5	12.80	278	331.14	*O*-galloylglucose III	$C_{13}H_{16}O_{10}$	332.16	331.16 (100); 270.80 (9.25), 211.84 (6.2), 169.12 (74.09), 125.01 (6.19)	[5, 24, 40–42]
7	14.42	280	497.24	*O*-methyl-di-*O*-galloylglucose	$C_{21}H_{22}O_{14}$	498.01	497.24 (5); 483.18 (100), 330.90 (17), 183.08 (44.21), 169.14 (34.8), 125.05 (7)	[40, 43]
13	21.72	280	787.23	Tetra-*O*-galloylglucose I	$C_{34}H_{28}O_{22}$	788.81	787.21 (100); 635.1 (7.2), 617.2 (2), 465.7 (3), 447.5 (5.0)	[5, 25, 40, 41, 43]
15	24.75	280	787.21	Tetra-*O*-galloylglucose II	$C_{34}H_{28}O_{22}$	788.18	787.21 (100); 635.32 (5), 617.2 (6), 465.1 (3.5), 447.5 (3.0)	[5, 25, 40, 41, 43]
16	25.78	280	787.31	Tetra-*O*-galloylglucose III	$C_{34}H_{28}O_{22}$	788.28	787.21 (100); 635.1 (17), 617.7 (15), 465.2 (4), 447.1 (2.0)	[5, 25, 40, 41, 43]
18	29.85	280	939.15	*Penta-*O*-galloylglucose	$C_{41}H_{32}O_{26}$	940.13	939.23 (100), 769.17 (12), 725.42 (2), 617.23 (5), 601.19 (2), 599.82 (2)	[5, 25, 40, 41, 43]
19	31.85	278	1091.1	Hexa-*O*-galloylglucose I	$C_{48}H_{36}O_{30}$	1092.07	1091.03 (100); 939.16 (32), 787.07 (3), 769.17 (6), 617.15 (3)	[5, 25, 40, 41, 43]
20	32.35	278	1091.3	Hexa-*O*-galloylglucose II	$C_{48}H_{36}O_{30}$	1092.20	1091.06 (100); 939.15 (33), 787.15 (3), 769.12 (9), 617.08 (4)	[5, 25, 40, 41, 43]
Gallic acid derivatives								
3	10.25	270	–	Gallic acid derivative	–	–	461.06 (100), 219.03 (83.23), 169.14 (27.3), 125.19 (10.15)	–
9	15.25	242, 280, 320	345.16	Gallic acid derivative	$C_{14}H_{18}O_{10}$	346.23	345.16 (100); 183.04 (49.01), 168.24 (16.79), 135.7 (7), 124.21 (10)	
11	16.87	270	493.21	Galloyl di-hexoside	$C_{19}H_{26}O_{15}$	494.86	493.21 (71.47); 313.68 (10.2), 222.97 (20), 169.02 (40.26)	[40, 43]

Table 2 (continued)

Peak no	Rt	UV ƛmax	[M-H]$^-$ m/z	Tentative identification	Formula	Molecular mass	HPLC-ESI (-)-MS^n experiment m/z (% base peak)	References
Benzoic acid derivative								
4	12.27	261	299.18	* *p*-Hydroxybenzoic acid hexoside	$C_{13}H_{16}O_8$	300.20	299.18 (100); 179.35 (10.1), [a]137.09 (15.52), [a]129.25 (2.1), [a]120.18 (3.2), [a]113.18 (2.5)	[40, 43]
Phaseic acid derivatives								
6	13.52	267	443.19	Dihydrophaseic acid hexoside I	$C_{21}H_{31}O_{10}$	444.22	443.19 (100); 425.34 (2), 237.77 (2), 219.07 (19.3), 160.9 (1)	[43]
8	14.87	267	443.25	Dihydrophaseic acid hexoside II	$C_{21}H_{31}O_{10}$	444.35	442.79 (100); 424.88 (5), 237.97 (7), 219.42 (27.3)	[43]
10	16.05	267	443.12	Dihydrophaseic acid hexoside III	$C_{21}H_{31}O_{10}$	444.50	443.19 (100); 425.02 (2), 237.01 (3), 219.24 (10.2), 161.7 (4.4)	[43]
Flavonoids								
12	20.25	241, 284, 350	–	*Quercetin derivative	–	–	[a]300.4 (24.52); [a]179.15 (10.2), [a]151.13 (13.2)	[44]
14	22.78	240, 282, 350	479.2	Myricetin hexoside	$C_{21}H_{20}O_{13}$	480.33	479.08 (100); 317.14 (14.5)	[45]
17	27.09	254, 320, 350	463.1	*Quercetin derivate	$C_{21}H_{20}O_{12}$	–	301.3 (5.38); [a]300.99 (100), 271.2 (4.5), 255.36 (6.5), [a]179.23 (5.4), [a]151.05 (3.2)	[44]
Standards								
Std	–	263	137.85	Hydroxybenzoic acid	$C_7H_6O_3$	138.91	MS^2[137.85];136.61 (10.2), 128.99 (2.1), 120.9 (3.1), 112.9 (1.9), 90.78 (100)	
Std	–	241, 274	939.15	Penta-*O*-galloylglucose	$C_{41}H_{32}O_{26}$	940.19	939.15; 787.31 (10), 769.16 (100), 725.14 (5), 617.25 (5), 601.29 (3), 599.20 (5), 465.21 (3) MS^2[939.15]: 787.31 (10), 769.16 (100),617.25 (5) MS^3[393.15–769.16]: 725.25 (18), 617.18 (100), 601.21 (24), 599.15 (34) MS^4[939.15–739.16–617.18]: 465	
Std	–	245, 266, 366	301.23	Quercetin	$C_{15}H_{10}O_7$	302.21	MS^2[301.23]: 179.12 (80), 151 (100)	

*Metabolites confirmed with MS experiments performed with authentic commercial standards. Benzoic acid and quercetin derivatives were identified using their nucleus molecule

Rt Retention time; Roman numerals: metabolites isomers

[a]*m/z* ions confirmed with the nucleus molecules standards. The rest of metabolites were identified by comparison of their fragmentation patterns with those previously identified in the literature. The references used for identification are cited in the corresponding column

Table 3 Changes in the levels of metabolites identified by UPLC-DAD-MS in the pulp of control and HW-treated 'Keitt' mango fruit after cold storage and ripening

Peak no	Metabolite	Treatment	Storage conditions		
			0 days at 5 °C	20 days at 5 °C	20 days at 5 °C + 7 days a 21 °C
Gallotannins (mg GAE/kg FW)					
O-galloylglucose					
1	*O*-galloylglucose I	Control	116.91 ± 1.91^{Aa}	58.19 ± 8.93^{Bb}	108.56 ± 17.01^{Ab}
		HWT	88.38 ± 5.20^{Cb}	109.86 ± 1.64^{Ba}	140.42 ± 0.59^{Aa}
2	*O*-galloylglucose II	Control	2.57 ± 0.13^{Ca}	3.93 ± 0.93^{Ba}	9.51 ± 1.28^{Aa}
		HWT	2.81 ± 0.47^{Ba}	3.51 ± 0.18^{Ba}	8.84 ± 0.70^{Aa}
5	*O*-galloylglucose III	Control	19.64 ± 3.68^{Ba}	13.33 ± 0.84^{Cb}	28.51 ± 2.78^{Aa}
		HWT	18.45 ± 0.00^{Ca}	22.44 ± 0.21^{Ba}	30.24 ± 0.38^{Aa}
Total content		Control	139.12 ± 5.58^{Aa}	75.32 ± 9.34^{Bb}	146.58 ± 15.03^{Ab}
		HWT	109.64 ± 4.97^{Cb}	135.81 ± 1.53^{Ba}	179.46 ± 0.87^{Aa}
Di-*O*-galloylglucose					
7	*O*-methyl-di-*O*-galloylglucose	Control	1.93 ± 0.08^{Cb}	3.72 ± 0.03^{Aa}	2.58 ± 0.37^{Bb}
		HWT	2.89 ± 0.03^{Aa}	3.26 ± 0.22^{Aa}	3.42 ± 0.43^{Aa}
Tetra-*O*-galloylglucose					
13	Tetra-*O*-galloylglucose I	Control	0.40 ± 0.04^{Ba}	0.97 ± 0.08^{Aa}	0.49 ± 0.12^{Bb}
		HWT	0.50 ± 0.06^{Ba}	0.66 ± 0.04^{Bb}	1.06 ± 0.10^{Aa}
15	Tetra-*O*-galloylglucose II	Control	0.47 ± 0.20^{Ba}	0.82 ± 0.01^{Aa}	0.74 ± 0.33^{ABb}
		HWT	0.53 ± 0.03^{Ba}	0.45 ± 0.06^{Bb}	1.10 ± 0.06^{Aa}
Total content		Control	0.87 ± 0.23^{Ba}	1.79 ± 0.09^{Aa}	1.23 ± 0.41^{Bb}
		HWT	1.03 ± 0.09^{Ba}	1.11 ± 0.08^{Bb}	2.16 ± 0.17^{Aa}
Penta-*O*-galloylglucose					
18	*Penta-*O*-galloylglucose	Control	2.65 ± 0.35^{Bb}	6.13 ± 0.61^{Aa}	5.58 ± 0.49^{Ab}
		HWT	5.09 ± 0.35^{Ba}	4.03 ± 0.54^{Cb}	6.68 ± 0.22^{Aa}
Hexa-*O*-galloylglucose					
19	Hexa-*O*-galloylglucose I	Control	0.85 ± 0.13^{Bb}	1.67 ± 0.11^{Aa}	1.76 ± 0.16^{Ab}
		HWT	1.71 ± 0.29^{Ba}	1.33 ± 0.14^{Ca}	2.14 ± 0.32^{Aa}
20	Hexa-*O*-galloylglucose II	Control	0.88 ± 0.19^{Bb}	2.28 ± 0.08^{Aa}	1.49 ± 0.44^{Ab}
		HWT	2.19 ± 0.33^{Ba}	1.40 ± 0.17^{Cb}	2.93 ± 0.33^{Aa}
Total content		Control	1.73 ± 0.32^{Bb}	3.95 ± 0.09^{Aa}	3.25 ± 0.39^{Ab}
		HWT	3.90 ± 0.62^{Ba}	2.73 ± 0.31^{Cb}	5.07 ± 0.66^{Aa}
Gallic acid derivative (mg GAE/kg FW)					
3	Gallic acid derivative	Control	10.41 ± 0.37^{Bb}	16.54 ± 2.24^{Aa}	11.50 ± 1.81^{Bb}
		HWT	14.68 ± 0.85^{Ba}	15.71 ± 0.85^{Ba}	22.48 ± 4.26^{Aa}
9	Gallic acid derivative	Control	0.72 ± 0.04^{Aa}	0.82 ± 0.11^{Aa}	0.89 ± 0.28^{Aa}
		HWT	0.55 ± 0.03^{Ba}	0.86 ± 0.13^{Aa}	0.63 ± 0.07^{Ba}
11	Galloyl di-hexoside	Control	0.91 ± 0.01^{Ba}	1.21 ± 0.26^{Bb}	2.14 ± 0.53^{Aa}
		HWT	0.97 ± 0.16^{Ba}	1.69 ± 0.18^{Aa}	1.10 ± 0.04^{Bb}
Benzoic acid derivatives (mg HAE/kg FW)					
4	**p*-Hydroxybenzoic acid hexoside	Control	3.25 ± 0.42^{Aa}	2.59 ± 0.26^{Bb}	2.94 ± 0.11^{ABa}
		HWT	2.30 ± 0.10^{Bb}	3.41 ± 0.45^{Aa}	3.34 ± 0.25^{Aa}
Phaseic acid derivatives (mg AAE/kg FW)					
6	Dihydrophaseic acid hexoside I	Control	1.78 ± 0.03^{Aa}	1.63 ± 0.25^{Ba}	1.50 ± 0.13^{Bb}
		HWT	1.25 ± 0.01^{Cb}	1.60 ± 0.17^{Ba}	1.87 ± 0.05^{Aa}
8	Dihydrophaseic acid hexoside II	Control	0.11 ± 0.02^{Aa}	0.09 ± 0.00^{Aa}	ND
		HWT	0.06 ± 0.01^{Aa}	0.08 ± 0.00^{Aa}	ND

Table 3 (continued)

Peak no	Metabolite	Treatment	Storage conditions		
			0 days at 5 °C	20 days at 5 °C	20 days at 5 °C + 7 days a 21 °C
10	Dihydrophaseic acid hexoside III	Control	0.21 ± 0.01^{Bb}	0.45 ± 0.03^{Aa}	0.24 ± 0.02^{Bb}
		HWT	0.34 ± 0.04^{Ba}	0.33 ± 0.00^{Bb}	0.39 ± 0.00^{Aa}
Total content		Control	2.1 ± 0.01^{Aa}	2.17 ± 0.27^{Aa}	1.74 ± 0.15^{Bb}
		HWT	1.65 ± 0.06^{Bb}	2.01 ± 0.17^{Aa}	2.26 ± 0.05^{Aa}
Flavonoids (mg QE/kg FW, mg ME/kg FW)					
12	*Quercetin derivative	Control	0.26 ± 0.03^{Aa}	0.34 ± 0.03^{Aa}	0.31 ± 0.06^{Ab}
		HWT	0.28 ± 0.14^{Ba}	0.42 ± 0.02^{Ba}	0.65 ± 0.08^{Aa}
17	*Quercetin derivative	Control	0.01 ± 0.00^{Ca}	0.07 ± 0.00^{Ba}	0.52 ± 0.03^{Aa}
		HWT	0.01 ± 0.00^{Ca}	0.05 ± 0.00^{Ba}	0.19 ± 0.00^{Ab}
Total content		Control	0.27 ± 0.03^{Ba}	0.41 ± 0.04^{Ba}	0.83 ± 0.09^{Aa}
		HWT	0.29 ± 0.14^{Ba}	0.47 ± 0.02^{Ba}	0.84 ± 0.08^{Aa}
14	Myricetin hexoside	Control	0.13 ± 0.01^{Ba}	0.13 ± 0.00^{Ba}	0.19 ± 0.03^{Aa}
		HWT	0.07 ± 0.07^{Cb}	0.13 ± 0.00^{Ba}	0.21 ± 0.02^{Aa}

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

HWT hot water treatment; *GAE* gallic acid equivalents; *HAE* hydroxybenzoic acid equivalents; *AAE* abscisic acid equivalents; *ME* myricetin equivalents; *QE* quercetin equivalents; *ND* not detected

Table 4 Total phenolic content and antioxidant activity of the pulp from control and HW-treated 'Keitt' mango fruit stored under chilling conditions and ripened

Treatment	Storage conditions		
	0 days at 5 °C	20 days at 5 °C	20 days at 5 °C + 7 days a 21 °C
Total phenolic (mg GAE/100 g FW)			
Control	30.6 ± 1.0^{Aa}	20.1 ± 3.5^{Ba}	23.3 ± 4.04^{Bb}
HWT	26.6 ± 2.4^{Ba}	23.3 ± 1.7^{Ba}	33.4 ± 2.3^{Aa}
ABTS (µmol TE/100 g FW)			
Control	112.1 ± 1.2^{Aa}	66.6 ± 10.0^{Ca}	85.9 ± 11.8^{Bb}
HWT	88.6 ± 11.2^{Bb}	77.6 ± 4.4^{Ba}	106.6 ± 8.8^{Aa}
DPPH (µmol TE/100 g FW)			
Control	123.8 ± 3.4^{Aa}	84.7 ± 5.8^{Ba}	80.2 ± 13.2^{Bb}
HWT	100.7 ± 4.1^{Ab}	88.9 ± 4.5^{Ba}	107.1 ± 5.9^{Aa}
FRAP (µmol TE/100 g FW)			
Control	66.7 ± 0.3^{Aa}	39.8 ± 1.6^{Ba}	38.8 ± 10.3^{Bb}
HWT	54.8 ± 7.4^{Ab}	48.2 ± 1.1^{Aa}	55.1 ± 4.7^{Aa}

Values are the mean ± SD of three replicates. GAE, gallic acid equivalents; QE, quercetin equivalents; TE, Trolox equivalents. Different letters in the same row (A, B, C) or column (a, b) indicate significant differences (Fisher, $P < 0.05$) between storage conditions and treatments, respectively

Principal component analysis

Data obtained from the evaluation of CI index, physiological (EL and MDA content) and quality parameters (firmness and color), quantification of metabolites by UPLC-DAD-MS, TP content, and antioxidant capacity (ABTS, DPPH and FRAP) were used to perform the principal component analysis (PCA) (Fig. 2). Two components explained 65.8% of the total variation (PC1 = 41.3% and PC2 = 24.5%) and the analysis separated the samples into six clusters according to the postharvest treatments and storage conditions (Fig. 2). The variables studied were grouped in three clusters as follows: cluster 1 in the lower right quadrant was formed by CI index and physiological parameters (EL and MDA) and was located close to control samples with severe CI symptoms (chilled and ripened) (Fig. 2). Cluster 2 in the upper right quadrant was formed by most of the metabolites identified by UPLC-DAD-MS (*e.g., O*-galloylglucose, tetra-*O*-galloylglucose, penta-*O*-galloylglucose, hexa-*O*-galloylglucose, etc.) and the pulp color parameters (a* and b*); this group was located close to the ripe HWT samples that had lower CI index values than those of control samples (20 days at 5 °C + 7 days at 21 °C) (Fig. 2). Finally, the third cluster was formed by the total TP content and the

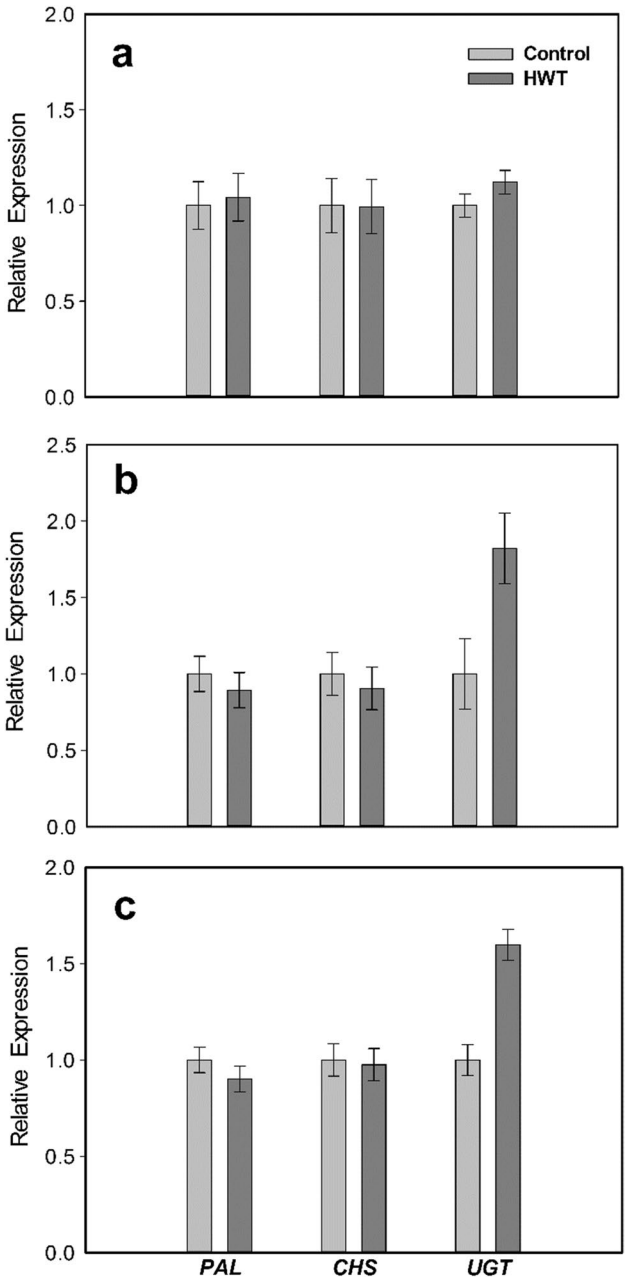

Fig. 1 Relative expression of genes encoding proteins differentially accumulated between control and HW-treated fruit before cold storage (0 d at 5 °C) (a), after cold storage (20 d at 5 °C) (b) and after ripening (20 d at 5 °C + 7 d at 21 °C) (C). The *ACT* gene was used as reference gene and the expression values are relative to that of the control fruit. Means are significantly different when the bars do not horizontally overlap. *PAL*, phenylalanine ammonium lyase; *CHS*, chalcone synthase; *UGT*, UDP-glucose:galloyl-1-*O*-β-D-glucosyltransferase

antioxidant activity (ABTS, DPPH, and FRAP). The variables were located in the upper left quadrant close to the non-chilled control sample (0 days at 5 °C) (Fig. 2).

Discussion

Quarantine HWT alleviates chilling injury in mango fruit

The lower CII values and less incidence of CI symptoms observed in HWT fruit (Table 1; Online Resource 1A) indicated that the heat treatment protected the fruit from the chilling stress. These results correspond with previous studies where the HWT was successfully used to induce CI tolerance in 'Keitt' mango [4, 6]. In addition, the lower values of the physiological parameters EL and MDA content in HWT fruit (Table 1) suggests a better integrity of the membranes that allow to maintain the cellular homeostasis [27], energy production [5, 11] and pigment synthesis [4], which favors normal ripening and development of the yellowish color in the pulp (Table 1, Online Resource 1B). We previously reported that the HWT-induced CI tolerance in mango fruit is associated with increased accumulation of the enzymes PAL, CHS, and chalcone isomerase (CHI) [6], and metabolites derived from gallic acid and flavonoids in the peel [5]. These mechanisms were further investigated in the present study comparing the metabolite profiles from the pulp of control and HW-treated fruit stored under chilling conditions and ripened.

Chilling injury tolerance induced by quarantine HWT is associated with the accumulation of gallic acid derivatives in mango pulp

The compound *O*-galloylglucose is the immediate precursor of gallotannins [28] and was the most abundant in the pulp of'Keitt' mango (Table 3), representing about 60% of the total phenolic content (Table 4). The lower accumulation of *O*-galloylglucose observed in HWT fruit before cold storage (0 days at 5 °C) can be associated with a decrease in the synthesis of this compound due to the heat stress [29] (Table 3). This effect could also be attributed to the synthesis of more complex molecules like gallotannins, which are synthesized by galloyltransferases whose activity is optimal at 44–45 °C [30], a temperature close to that of the HWT. In this sense, the reduction in the content of *O*-galloylglucose in HWT fruit corresponded with higher levels of *O*-methyl-di-*O*-galloylglucose, penta-*O*-galloylglucose, hexa-*O*-galloylglucose and one gallic acid derivative (compound 3) (Table 3). These results agree with those of Vega-Alvarez et al. [5] who observed that quarantine HWT increased the levels of these complex gallotannins in the peel of 'Keitt' mango fruit. Kim et al. [31] reported that the immersion of mango cv. Tommy Atkins (497.5 g) in hot water (46.1 °C, 70 min) also increased the levels of gallotannins in the pulp.

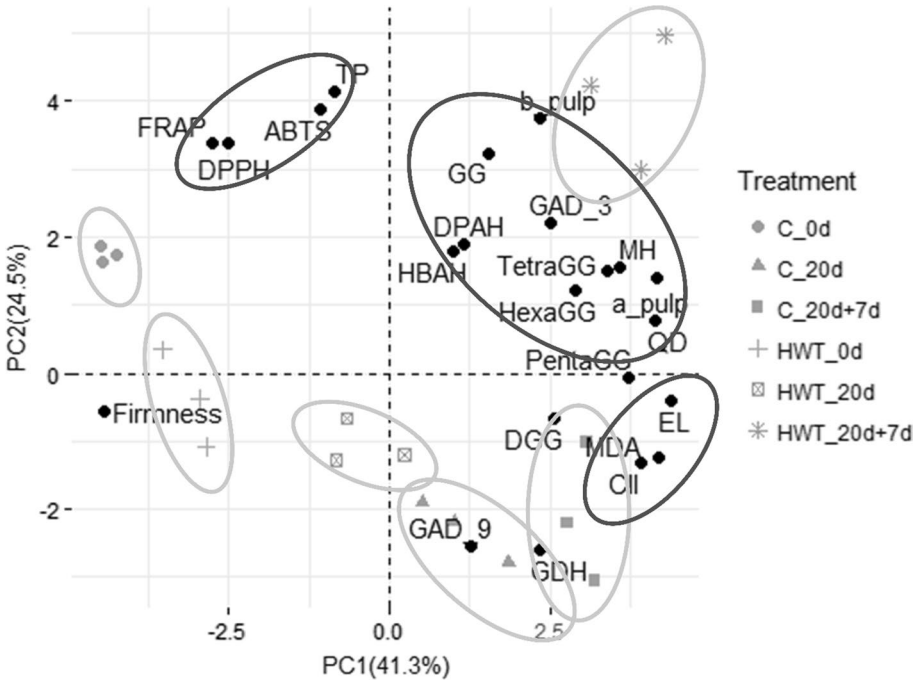

Fig. 2 A biplot based on principal component analysis of CI index, physiological and quality parameters, metabolites identified by UPLC-MS, TP content and the antioxidant capacity of the pulp from control (C) and hot water treated (HWT) mango cv. 'Keitt' stored for 0 d at 5 °C (0 d), 20 d at 5 °C (20 d), and 20 d at 5 °C plus 7 d at 21 °C (20 d + 7 d). Samples are represented by colored-shapes and the variables by black points. Chilling injury index (CII), malondialdehyde content (MDA), electrolyte leakage (EL), firmness, pulp color parameters (a* and b*), galloylglucose (GG), *O*-methyl-di-O-galloyl glucose (DGG), tetra, penta, hexa-*O*-gallotannins (GG), gallic acid derivatives (GAD_9 and GAD_3), galloyl di-hexoside (GDH), dihydrophaseic acid hexoside (DPAH), quercetin derivative (QD), myricetin hexoside (MH), hydroxybenzoic acid hexoside (HBAH), total phenolics (TP), and antioxidant capacity (ABTS, DPPH and FRAP)

Gallic acid is synthesized from the shikimate pathway intermediate 3-dehydroshikimate whereas *p*-hydroxybenzoic acid and myricetin are derived from phenylalanine, an amino acid produced from chorismate, the final product of the shikimate pathway [32]. Thus, the reduction in the levels of *p*-hydroxybenzoic hexoside acid and myricetin hexoside by the HWT before cold storage (0 days at 5 °C) (Table 3) could be associated with a greater flux of metabolites towards the synthesis of gallic acid, which is the base molecule of gallotannins.

Chilling stress caused an adjustment in the secondary metabolism of the pulp in both control and HWT fruit (20 days at 5 °C). In control fruit there was an increase in the accumulation of gallotannins and a decrease in the levels of *O*-galloylglucose (the most abundant metabolite) and *p*-hydroxybenzoic acid hexoside by 45% and 20%, respectively (Table 3). These results corresponded with those of Rosalie et al. [33] who reported that the storage of mango fruit 'Cogshall' at 7 °C decreased the content of galloyl-*O*-glycoside in the pulp. Cold storage may have affected the function of the enzyme that synthesizes *O*-galloylglucose whose activity was previously shown to decrease up to seven times after lowering the temperature from 10 to 0 °C [29]. The HWT mitigated the negative impact of the chilling stress more likely due to the action of heat shock proteins (HSP), which were shown to be induced by this treatment in the peel of 'Keitt' mango [6]. HSP can stabilize key enzymes of the secondary metabolism and maintain higher levels of metabolites as observed in the pulp of HWT fruit after cold storage (20 days at 5 °C) and after ripening (20 days at 5 °C + 7 days at 21 °C) (Table 3). In addition, the higher content of *O*-galloylglucose in HWT fruit corresponded with the up-regulation of *UGT* (Fig. 1), which encodes the enzyme glucosyltransferase that synthesizes this metabolite (Fig. 1B and C). These results suggest that glucosyltransferase plays a key role in the acquisition of chilling tolerance in HWT fruit by increasing the levels of *O*-galloylglucose to counteract the cold stress. In this sense, Vega-Alvarez et al. [5] reported that the CI tolerance induced by the quarantine HWT in 'Keitt' mango was associated with increased accumulation of gallic acid derivatives in the peel, specifically gallotannins.

Dihydrophaseic acid is the main product from the catabolism of abscisic acid (ABA), a hormone that plays an important role in fruit development and the protection against abiotic and biotic stresses [34]. The exogenous application of ABA alleviates chilling injury in climacteric fruit, effect associated mainly with an enhancement

of the ROS scavenging system [35]. The higher levels of dihydrophaseic acid hexose observed in HWT fruit after ripening (Table 3) were also reported in HW-treated tomatoes after cold storage and ripening [36]. The lower levels of this compound in control fruit may be associated with a reduced catabolism of ABA to counteract the chilling stress.

The total content of quercetin derivatives and myricetin hexoside increased significantly after the ripening period in both treatments, but their values were similar between control and HWT fruit (Table 3). These results correspond with the similar expression levels of *PAL* and *CHS* observed in the same samples (Fig. 1). The metabolite profiles suggest that the alleviation of chilling injury in HWT fruit is mediated by the enhancement of the ROS scavenging system through an increased accumulation of gallic acid derivatives during chilling stress and ripening as previously shown in mango peel [5].

HWT improves the antioxidant capacity in mango pulp

HWT fruit showed higher AC values than control fruit after cold storage and ripening (Table 4). The AC appears to be associated mainly with the TP content as suggested by the positive correlation found between these parameters (ABTS, $r = 0.82$, $P < 0.001$; DPPH, $r = 0.70$, $P < 0.01$; FRAP, $r = 0.76$, $P < 0.001$). In particular, HWT fruit showed greater accumulation of *O*-galloylglucose and gallotannins (Table 3) associated with higher expression of the *UGT* gene (Fig. 1). In this sense, previous studies have reported high AC values for gallic acid derivatives [37–39]. Thus, the chilling tolerance in HWT fruit appears to be associated with a better capacity to control the oxidative stress as previously suggested for'Shelly' mangoes [9].

Variables associated with chilling injury tolerance and susceptibility

PCA analysis allowed the association of the CI index, physiological and pulp color parameters, the metabolites identified by UPLC-DAD-MS, the TP content, and AC data with the chilling injury tolerance and susceptibility (Fig. 2). Ripe HWT samples were located in the upper right quadrant, very close to gallic acid derivatives (*O*-galloylglucose, tetra-*O*-galloylglucose, penta-*O*-galloylglucose, hexa-*O*-galloylglucose and compound 3), dihydrophaseic acid hexoside and pulp color parameters (a* and b*) (Fig. 2). In addition, these samples were located closer to TP content and AC (ABTS, DPPH and FRAP) parameters than chilled and ripened control fruit with severe CI symptoms (Fig. 2). These results support the idea that chilling tolerance in HW-treated fruit is associated with the control of oxidative stress by increasing the levels of antioxidant metabolites like gallic acid derivatives as previously reported in the peel of'Keitt' mango [5]. On the other hand, chilled ripe control samples located in the lower right quadrant were clustered with CI index and physiological parameters (EL and MDA content), indicating that chilling stress and ripening had a strong negative impact on the membrane integrity, leading to more severe CI symptoms. Thus, chilling injury development is related to a decrease in membrane functionality as suggested previously in mango [4, 6, 7].

Conclusion

The chilling injury tolerance induced by HWT in 'Keitt' mango fruit is associated with an increase in the synthesis of gallotanins in the pulp, highlighting *O*-galloylglucose that accounts for 60% of the total phenolic content, which is associated with a higher antioxidant capacity that may protect against the oxidative stress induced by cold storage and ripening. This information extends the understanding of the mechanisms involved in CI tolerance in mango fruit and provides targets that could be used to develop strategies for the prevention of this disorder (e.g., selection and development of CI tolerant cultivars). The application of these strategies will increase the economic value and decrease postharvest losses. Further studies measuring the activity of enzymes involved in the synthesis of gallotanins will confirm the role of these compounds in cold tolerance.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11694-023-01866-z.

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Author Contributions KVPH: Data curation, Investigation, Writing-original draft. EVA: Data curation, Investigation, Writing-original draft. AVA: Investigation, Methodology. NYSS: Methodology (UPLC-DAD-MS). CIJB: Methodology (RT-qPCR). JALV: Conceptualization, Supervision, Writing-Reviewing and Editing.

Data Availability All data generated or analysed during this study are included in this published article (and its supplementary information files).

Declarations

Conflict of interest The authors have no conflict of interest to declare.

References

1. SIAP, Panorama Agroalimentario 2021. (Servicio de Información Agroalimentaria y Pesquera, 2021), https://nube.siap.gob.mx/gobmx_publicaciones_siap/pag/2021/Panorama-Agroalimentario-2021. Accessed 3 Oct 2022
2. FAOSTAT, Crops and livestock products. (Food and Agriculture Organization of the United Nations, 2022), https://www.fao.org/faostat/es/#data/QCL/visualize. Accessed 3 Oct 2022
3. SADER, El mango, producto estrella en México. (Secretaría de Agricultura y Desarrollo Rural, 2022), https://www.gob.mx/agricultura/articulos/el-mango-producto-estrella-en-mexico. Accessed 3 Oct 2022
4. M. López-López, J.A. López-Valenzuela, F. Delgado-Vargas, G. López Angulo, A. Carrillo-Lopez, L.E. Ayón-Reyna et al., HortScience **53**, 217–223 (2018). https://doi.org/10.21273/HORTSCI12575-17
5. M. Vega-Alvarez, N.Y. Salazar-Salas, G. López-Angulo, K.V. Pineda-Hidalgo, M.E. López-López, M.O. Vega-García et al., Postharvest Biol. Technol. **169**, 111299 (2020). https://doi.org/10.1016/j.postharvbio.2020.111299
6. N.Y. Salazar-Salas, D.A. Chairez-Vega, M. Vega-Alvarez, D.G. González-Nuñez, K.V. Pineda-Hidalgo, J. Chávez-Ontiveros et al., Postharvest Biol. Technol. **186**, 111838 (2022). https://doi.org/10.1016/j.postharvbio.2022.111838
7. V. Sivankalyani, N. Sela, O. Feygenberg, H. Zemach, D. Maurer, N. Alkan, Front. Plant Sci. **7**, 1579–1579 (2016). https://doi.org/10.3389/fpls.2016.01579
8. V. Sivankalyani, I. Maoz, O. Feygenberg, D. Feygenberg, N. Alkan, J. Agric. Food Chem. **65**(3), 632–638 (2017). https://doi.org/10.1021/acs.jafc.6b04355
9. P.K. Sudheeran, O. Feygenberg, D. Maurer, N. Alkan, Molecules **23**(7), 1832 (2018). https://doi.org/10.3390/molecules23071832
10. D. Cantre, E. Herremans, P. Verboven, J. Ampofo-Asiama, M.L.A.T.M. Hertog, B.M. Nicolaï, Postharvest Biol. Technol. **125**, 99–111 (2017). https://doi.org/10.1016/j.postharvbio.2016.11.009
11. P. Li, X. Zheng, Y. Liu, Y. Zhu, Food Chem. **142**, 72–78 (2014). https://doi.org/10.1016/j.foodchem.2013.06.132
12. Z. Zhao, J. Cao, W. Jiang, Y. Gu, Y. Zhao, J. Sci. Food Agric. **89**(2), 304–309 (2009). https://doi.org/10.1002/jsfa.3443
13. S. Kondo, K. Sungcome, S. Setha, N. Hirai, J. Hortic. Sci. Biotechnol. **79**(6), 891–896 (2004). https://doi.org/10.1080/14620316.2004.11511862
14. S. Nair, Z. Singh, S.C. Tan, J. Hortic. Sci. Biotechnol. **78**(6), 866–873 (2003). https://doi.org/10.1080/14620316.2003.11511711
15. USDA-APHIS, Quarantine Treatments. (Animal and Plant Health Inspection Service, U.S. Department of Agriculture, 2021), https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/sa_quarantine_treatments/ct_quarantine-treatment. Accessed 15 Jan 2022
16. D.A. Díaz-Corona, M.E. López-López, L.E. Ayón-Reyna, J.G. López-Velázquez, B.A. López-Zazueta, M.O. Vega-García, J. Food Biochem. **44**(8), e13286 (2020). https://doi.org/10.1111/jfbc.13286
17. E. Mitcham, R. McDonald, J. Am. Soc. Hortic. Sci. **117**(6), 919–924 (1992). https://doi.org/10.21273/JASHS.117.6.919
18. D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange, Planta **207**(4), 604–611 (1999). https://doi.org/10.1007/s004250050524
19. V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Methods Enzymol. **299**, 152–178 (1999). https://doi.org/10.1016/S0076-6879(99)99017-1
20. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radic. Biol. Med. **26**(9–10), 1231–1237 (1999). https://doi.org/10.1016/s0891-5849(98)00315-3
21. W. Brand-Williams, M.E. Cuvelier, C. Berset, LWT-Food Sci. Technol. **28**(1), 25–30 (1995). https://doi.org/10.1016/S0023-6438(95)80008-5
22. I.F. Benzie, J.J. Strain, Anal. Biochem. **239**(1), 70–76 (1996). https://doi.org/10.1006/abio.1996.0292
23. S. Chang, J. Puryear, J. Cairney, Plant Mol. Biol. Rep. **11**(2), 113–116 (1993). https://doi.org/10.1007/BF02670468
24. M. Sobeh, S. Rezq, O.M. Sabry, M.A.O. Abdelfattah, M.A. El Raey, W.A. El-Kashak et al., Biomed. Pharmacother. **115**, 108882 (2019). https://doi.org/10.1016/j.biopha.2019.108882
25. N. Berardini, R. Carle, A. Schieber, Rapid Commun. Mass Spectrom. **18**, 2208–2216 (2004). https://doi.org/10.1002/rcm.1611
26. M.-J. Wu, X.-L. Wu, D.-Q. Zhang, F. Qiu, L.-Q. Ding, H.-L. Ma et al., Biomed. Chromatogr. **31**(12), e4016 (2017). https://doi.org/10.1002/bmc.4016
27. J. Yang, M.-R. Fu, Y.-Y. Zhao, L.-C. Mao, Agric. Sci. China **8**(3), 304–310 (2009). https://doi.org/10.1016/S1671-2927(08)60213-8
28. K.A. Krenek, R.C. Barnes, S.T. Talcott, J. Agric. Food. Chem. **62**(39), 9515–9521 (2014). https://doi.org/10.1021/jf5031554
29. Q. Ye, S. Zhang, N. Qiu, L. Liu, W. Wang, Q. Xie et al., Molecules **26**(15), 4650 (2021). https://doi.org/10.3390/molecules26154650
30. B. Fröhlich, R. Niemetz, G. Gross, Planta **216**(1), 168–172 (2002). https://doi.org/10.1007/s00425-002-0877-3
31. Y. Kim, A. Lounds-Singleton, S. Talcott, Food Chem. **115**, 989–993 (2009). https://doi.org/10.1016/j.foodchem.2009.01.019
32. J. Widhalm, N. Dudareva, Mol. Plant **8**(1), 83–97 (2015). https://doi.org/10.1093/mp/ssu126
33. R. Rosalie, J. Joas, C. Mertz, L. Dufossé, M. Léchaudel, Plants **11**(22), 3038 (2022). https://doi.org/10.3390/plants11223038
34. K. Gupta, S. Wani, A. Razzaq, M. Skalicky, K. Samantara, S. Gupta et al., Front. Plant Sci. **13**, 1–20 (2022). https://doi.org/10.3389/fpls.2022.817500
35. J. Tang, Y. Zhao, S. Qi, Q. Dai, Q. Lin, Y. Duan, Front. Plant Sci. **13**, 987573 (2022). https://doi.org/10.3389/fpls.2022.987573
36. F. Delgado-Vargas, M. Vega-Alvarez, A. Landeros Sanchez, G. Lopez-Angulo, N.Y. Salazar-Salas, M.F. Quintero-Soto et al., J. Food Biochem. **46**(2), e14056 (2022). https://doi.org/10.1111/jfbc.14056
37. N. Bouchet, L. Barrier, B. Fauconneau, Phytother. Res. **12**(3), 159–162 (1998). https://doi.org/10.1002/(SICI)1099-1573(199805)12:3%3c159::AID-PTR209%3e3.0.CO;2-C
38. L.Y. Jiang, S. He, C. Sun, Food Chem. **119**(4), 1285–1292 (2010). https://doi.org/10.1016/j.foodchem.2009.09.005
39. R.Y. Gan, K.W. Kong, H.B. Li, K. Wu, Y.Y. Ge, C.L. Chan et al., Front. Chem. **6**, 1–39 (2018). https://doi.org/10.3389/fchem.2018.00039
40. A.M. Gómez-Caravaca, A. López-Cobo, V. Verardo, A. Segura-Carretero, A. Fernández-Gutiérrez, Electrophoresis **37**(7–8), 1072–1084 (2016). https://doi.org/10.1002/elps.201500439
41. J.T. Pierson, G.R. Monteith, S.J. Roberts-Thomson, R.G. Dietzgen, M.J. Gidley, P.N. Shaw, Food Chem. **149**, 253–263 (2014). https://doi.org/10.1016/j.foodchem.2013.10.108
42. J.E. Ramirez, R. Zambrano, B. Sepúlveda, M.J. Simirgiotis, Molecules **19**(1), 438–458 (2014). https://doi.org/10.3390/molecules19010438
43. M. Navarro, E. Arnaez, I. Moreira, S. Quesada, G. Azofeifa, K. Wilhelm et al., Foods **8**(9), 384 (2019). https://doi.org/10.3390/foods8090384
44. A. Schieber, N. Berardini, R. Carle, J. Agric. Food Chem. **51**(17), 5006–5011 (2003). https://doi.org/10.1021/jf030218f
45. L. Tan, Z. Jin, Y. Ge, H. Nadeem, Z. Cheng, F. Azeem et al., Sci. Rep. **10**(1), 20017 (2020). https://doi.org/10.1038/s41598-020-75636-y