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Article

Postharvest treatment with methyl jasmonate impacts lipid metabolism in tomatoes (*Solanum lycopersicum* L. cv. Grape) at different ripening stages

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Abstract: Application of exogenous jasmonate can stimulate the production of ethylene, carotenoids and aroma compounds, resulting in the acceleration of fruit ripening. These alterations improve fruit quality and make fruit desirable for human consumption, but overripening of a fruit results in large losses of fruit crops. In order to overcome this problem, 1-methylcyclopropene was applied to the fruits due to its capacity to block the receptors of ethylene, resulting in the suppressed of fruit ripening. In this study, treatments only with 1-methylcyclopropene, and with both 1-methylcyclopropene and methyl jasmonate was conducted to observe if an exogenous methyl jasmonate can improve the levels of metabolites in their fruits with ethylene receptors blocked. Fruits were analyzed at 4, 10 and 21 day after harvest (DAH) and compared with the no treated fruits. The postharvest treatments affected primary metabolites (sugars, organic acids, amino acids and fatty acids) and secondary metabolites (carotenoids, tocopherols and phytosterols). However, the lipid metabolism of the tomato was the most impacted by the exogenous jasmonate. Fatty acids, carotenoids, tocopherols and phytosterols showed a delay in their production at 4 and 10 DAH. In contrast, at 21 DAH these non-polar metabolites exhibited an important improvement in their accumulation.

Keywords: postharvest treatment; jasmonate; metabolite profiling; lipid metabolism; *Solanum lycopersicum*; ethylene inhibition; fruit quality

1. Introduction

At the onset of tomato ripening, changes in primary metabolites were observed such as accumulation in glucose and fructose, and presence of citric and malic acids in ripe fruits [1]. Sugars and organic acids are critical to good flavor, contribute for sweetness and acid balance and, consequently, they are responsible for the consumer acceptance [2].

In addition changes in secondary metabolites of tomato fruit are observed mainly with those related to health benefits as well as lycopene, β-carotene, α-tocopherol, β-tocopherol and β-sitosterol. Carotenoids and tocopherols play an important role in human nutrition mainly due to antioxidant properties, and visual perception of ripe fruits, while phytosterols are associated in reducing LDL cholesterol and total cholesterol [3,4,5]. Many of these ripening processes are regulated by plant hormones such as ethylene, methyl jasmonate, abscisic acid and other phytohormones [6,7].

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Methyl jasmonate can interact with other phytohormones such as ethylene in promoting biological activity such as antibacterial and antifungal activities and signaling plant defenses [8]. Application of exogenous jasmonate stimulates the production of ethylene, degradation of chlorophyll, accumulation of β-carotene and production of aroma compounds, which can result in the acceleration of fruit ripening [9].

Although, these changes can improve the quality of the fruit, making it desirable for consumption, the overripening of the fruit can result in large losses of fruit crops. In order to overcome this problem, exogenous application of 1-methylcyclopropene in tomato fruits can be used due to its ability to reduce ethylene production and respiration rate of climacteric fruits [10]. This action prolongs the shelf-life of tomato fruits by retaining firmness, delaying lycopene production and consequently color development [11,12]. In this study, we investigated the metabolic response to methyl jasmonate in tomato fruits with ethylene inhibited by 1-methylcyclopropene during fruit ripening.

2. Materials and Methods

2.1 Plant material and postharvest treatment

Tomatoes (*Solanum lycopersicum* cv. Grape) in mature green stage (N = 1200) were collected from a commercial standard greenhouse in Ibiúna (23°39'21" S; 47°13'22" W), São Paulo, Brazil. Fruits were sterilized with 0.1 % sodium hypochlorite aqueous solution during 15 minutes. Four biological replicates were applied in the experiment and each of them were composed by 100 fruits. Tomatoes were randomly separated into three groups (N = 400 by group): 1) control group (CTRL), without any treatment; 2) treated 1-methylcyclopropene group (MCP); and 3) treated both 1-methylcyclopropene and methyl jasmonate group (MCP+MeJA). Fruits were left to ripen spontaneously in a 323 L chamber at constant temperature (20 ± 2 ºC) and humidity (80 ± 5% RH) in a 16-hour-day/8-hour-night cycle. For MCP group, 1-methylcyclopropene solution (100 ppm) was applied by syringe on the chamber for evaporation. For the MCP+MeJA group, methyl jasmonate solution (100 ppm) was applied in a filter paper left on the wall of the chamber for evaporation and 1-methylcyclopropene solution (100 ppm) as described for the MCP group. The methyl jasmonate and 1-methylcyclopropene applications were conducted for the second time after 12 hours of first exposition to the hormone, totalizing 24 hours of treatment. Samples of 10 fruits from each replicate were randomly taken at 4, 10 and 21 days after harvest (DAH), considering the control group as reference. Samples were frozen in liquid nitrogen and stored at -80 °C for subsequent analyses.

2.2 Ripening parameters

2.2.1 Ethylene emission

Ethylene emission was performed by placed five tomato fruits in airtight glass containers of 600 mL at 25 °C for 1h. Then, five samples of 1 mL of gas produced in the headspace were collected with gastight syringes through a rubber septum. A gas chromatography with a flame-ionization detector (GC-FID) (Agilent Technologies, HP-6890) and HP-Plot Q column (30 m x 0.53 mm x 40 μm) were used to evaluate ethylene emission. Temperatures of injector and detector were equally established at 250 °C, and the oven at 30 °C. The helium gas flow was set at 1 mL.min-1 and the injections were performed in pulsed splitless mode.

2.2.2 Fruit surface color

Fruit surface color measure was conducted using the colorimeter HunterLab ColorQuest XE instrument (Hunter Associates Laboratories) measure in terms of L*, a* and b* space. The experimental data were treated to obtain values of °hue angle. Three measurements were made at equator of six tomato fruits [13].

2.3 Analysis of metabolite profiling of tomato fruit using GC–MS

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2.3.1 Extraction and derivatization of polar metabolites

The extraction and derivatization of polar metabolites were conducted as described by [14]. For the extraction process 100 mg of frozen pericarp powder was mixed with an 100% distilled methanol at –20 °C (1400 µL) and ribitol (200 µg.mL-1, internal standard) (60 µL). The mixture was vortexed, incubated in a thermomixer at 950 rpm for 10 min at 70 °C, centrifuged at 11000 g for 10 min, and the supernatant collected. In the upper phase was added chloroform at –20 °C (750 µL) and Milli-Q water (1500 µL), following of mixture and centrifugation at 2200 g for 15 min. The upper hydrophilic phase (150 µL) were collected and dried under nitrogen gas. The derivatization of samples consisted in the addition of 20 mg.mL^{-1} metoxyamine hydrochloride (Sigma–Aldrich Chemical Co. St. Louis, MO, USA) (40 µL) and pyridine with subsequent incubation in an orbital shaker at 1000 rpm and 37 °C for 2 h. Consecutive, N-methyl-N-(trimethylsilyl) tri-fluoroacetamide (MSTFA) (70 µL) was added to the sample and incubated in an orbital shaker at 1000 g and 37 °C for 30 min. Finally, the derivatized samples were moved into glass vials and run on the GC-MS. A pool of polar metabolite external standards (1 mg.mL^{-1}, Sigma–Aldrich) was applied in order to certify the identified metabolites by mass spectra comparison: D-glucose; D-fructose; maltose; sucrose; D-galactose; myo-inositol; citric acid; L-alanine; L-serine; L-proline; L-aspartate; L-glutamate [15].

2.3.2 Extraction and derivatization of non-polar metabolites

For the extraction process 1000 mg of frozen pericarp powder was mixed with chloroform (1250 µL), methanol (2500 µL), n-tridecane (800 µg.mL^{-1}, internal standard) (20 µL), following of vortex for 10 s and incubation on ice for 30 min. Then 1.5% sodium sulfate (1250 µL) and chloroform (1250 µL) were added to the mixture, incubated on ice for 5 min and centrifuged at 4 °C for 1000 g and 15 min. The upper polar phase was collected and dried under nitrogen gas. The sample was redissolved in hexane (1000 µL), toluene (200 µL), methanol (1500 µL) and 8% cloridric acid (300 µL), mixed for 10 s and incubated for 1.5h at 100 °C. After that, hexane (1000 µL) and Milli-Q water was added to the sample and mixed [16,17,18]. The hexane phase was separated and dried under nitrogen gas. The sample was redissolved in hexane (80 µL) and pyridine (20 µL), and derivatized with MSTFA (40 µL). Finally, the derivatized samples were moved into glass vials and run on the GC-MS. A pool of fatty acid methyl esters (FAME) external standards (Sigma–Aldrich) was applied in order to certify the identified metabolites by mass spectra comparison: methyl laurate (C12:0, 0.8 mg.mL^{-1}); methyl tetradecanoate (C14:0, 0.8 mg.mL^{-1}); methyl palmitate (C16:0, 0.8 mg.mL^{-1}); methyl octadecanoate (C18:0, 0.4 mg.mL^{-1}); methyl arachidate (C20:0, 0.4 mg.mL^{-1}); methyl docosanoate (C22:0, 0.4 mg.mL^{-1}); methyl lignocerate (C24:0, 0.4 mg.mL^{-1}); methyl linoleate (C 18:2, 0.4 mg.mL^{-1}); (Z) -9-oleyl methyl ester (C 18:1, 0.4 mg.mL^{-1}); methyl linolenate (C 18:3, 0.4 mg.mL^{-1}) and methyl palmitoleate (C 16:1, 0.8 mg.mL^{-1}) [15].

2.3.3 GC-MS analysis

Derivatized samples were evaluated on a gas chromatography–mass spectrometry (Agilent GC-MS 5977, Agilent Technologies, CA, USA) [15]. Trimethylsilyl derivatives (1 µL) was injected into an injector at 230 °C and split-less mode. The oven temperature ramp applied was 80 °C (initial temperature), held for 2 min, heating at 15 °C.min^{-1} to 330 °C and held for 6 min. The electron impact ionization mass spectrometer was settled to: 70 eV of ionization voltage; 250 °C of ion source temperature; 250 °C of injection port temperature; 70–600 m/z at 20 scans.s^{-1} of mass scan range. The column used was a HP5ms column (30 m x 0.25 m x 0.25 µm). The flow rate of helium gas was 2 mL.min-1. Acquisition, deconvolution, and analyses of experimental data were processed by Mass Hunter software (Agilent, CA, EUA). For retention index (RI) comparison and data validation was used the NIST mass spectral library (NIST 2011, Gaithersburg, MD, USA). Some of the identified metabolites were also confirmed by mass spectral comparison with the authentic external standards previously described.

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2.4 Analysis of carotenoids by HPLC

For the extraction of carotenoids frozen pericarp powder (200 mg) was mixed with 100 µL of 30% NaCl (w:v) solution and 200 µL of dichloromethane. Hexane:ether (1:1) (500 µL) was added to the mixture and centrifuged (13000 g at 4 ◎C for 5 min). This protocol was repeated three times and the organic phases were pooled together [19]. The upper phase was dried under nitrogen gas and dissolved in ethyl acetate. The HPLC (Infinity 1260 HPLC, Agilent Technologies, USA) was coupled to a diode array detector (DAD) equipped with YMC Carotenoid HPLC C30 (5 µm x 250 mm x 4.6 mm) column [20]. Lycopene, β-carotene and lutein from Sigma–Aldrich were used as standards.

2.5 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD) of four biological replicates. Statistical analysis was performed by one-way analysis of variance (ANOVA) and Tukey´s test was applied to establish significant differences among mean values at $P < 0.05$, using the Minitab 19.0 software package. For multivariate analysis, raw data were normalized by internal standard area, processed using log transformation (log 2) and mean-centered and divided by the square root of deviation of each variable (Pareto scaling). Principal Component Analysis (PCA), heatmaps and fold change analysis were executed to evaluate differences between treated and no-treated groups, using the Metaboanalyst 4.0 server [21].

3. Results and Discussion

3.1 Effect of methyl jasmonate on the ethylene emission and fruit surface color in tomatoes

In this study, the alterations of metabolites identified in tomato fruits under post-harvest treatments were observed. Therefore, one group of fruits with ethylene inhibited by 1-methylcyclopropene were exposed to methyl jasmonate hormone (MCP+MeJA), other group of fruit were treated only with 1-methylcyclopropene (MCP) and the no treated tomato fruits (CTRL) were used as reference of the assays. The three groups of fruits were possible to visualize in the Figure 1A.

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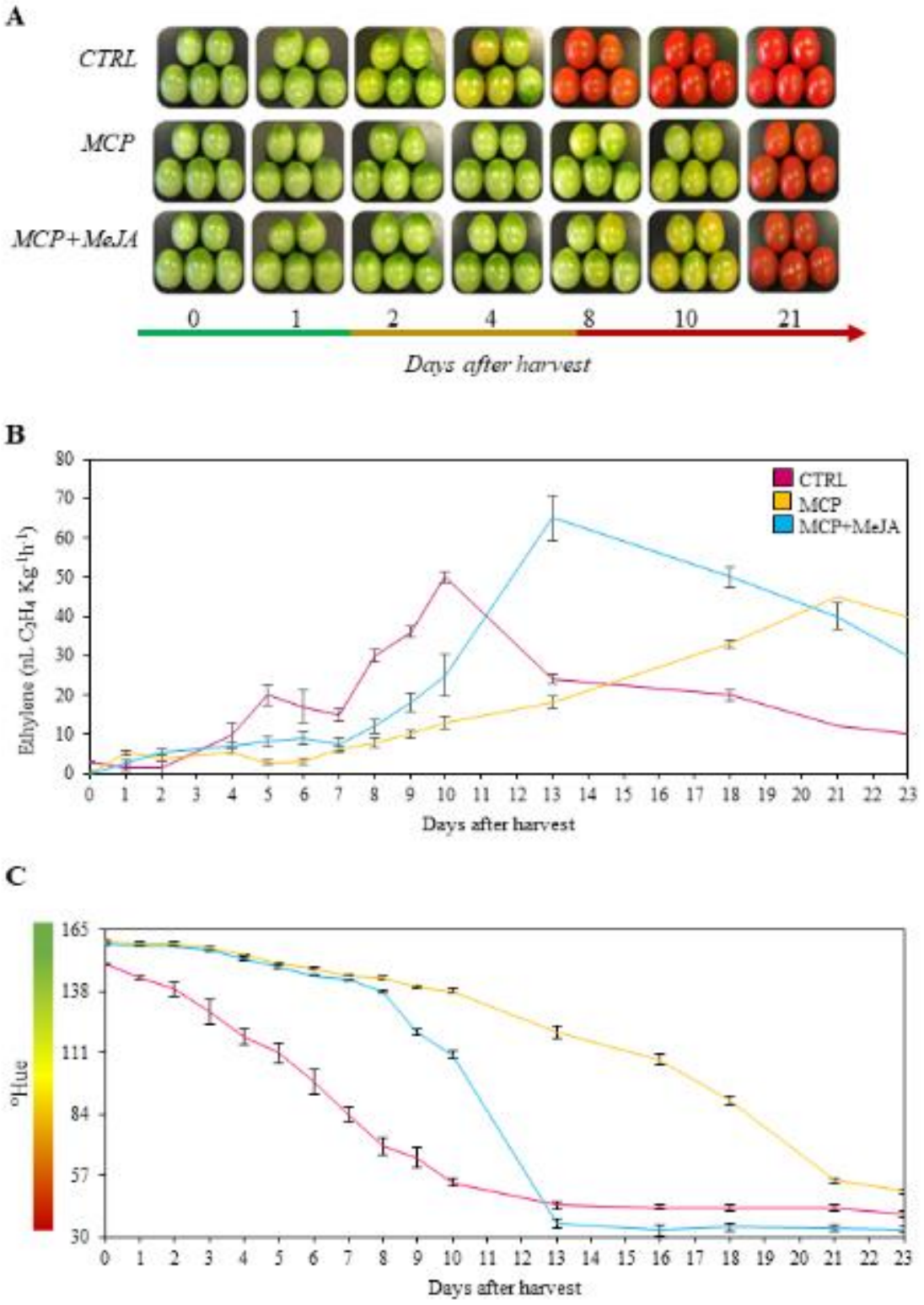

Figure 1. Characterization of tomato (*Solanum lycopersicum* L. cv. Grape) fruits treated with 1-methylcyclopropene (MCP) and both hormones 1-methylcyclopropene and methyl jasmonate (MCP+MeJA) during ripening. Representative images of tomatoes (A). Effects of MCP and MCP+MeJA on ethylene emission (B) and fruit color (C) compared to the control group (CTRL). Values are means ± standard error of four biological replicates of at least 10 fruits each.

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The CTRL group fruits achieved the breaker stage at 4 DAH and ripe stage at 10 DAH. Regarding treated fruits, breaker and red stages were achieved by MCP at 13 and 21 DAH, respectively, while MCP+MeJA at 10 and 13 DAH, respectively. For the characterization of ripening stages of the CTRL group, measures of ethylene emission and surface color of the tomato fruits were realized from the day of harvest to 21 DAH (Figure 1B and 1C). While the analysis of metabolite profiling were realized at 4, 10 and 21 DAH, aiming to observe the effect of treatments with respect to CTRL.

Treatments with both 1-methylcyclopropene and methyl jasmonate, and only 1-methylcyclopropene showed a delay in fruit ripening by the reduction of ethylene emission and fruit surface color, when compared with CTRL group. A similar result was observed in the study in which the tomato was treated with 1-methylcyclopropene and reported a reduction in ethylene emission and respiration rate [22]. Both groups MCP and MCP+MeJA presented the characteristics curves of ethylene emission of climacteric fruits. Fruits treated only with 1-methylcyclopropene showed the longest delay in fruit ripening, which were characterized by its peak ethylene and redness color at 21 DAH. While, tomatoes treated with both 1-methylcyclopropene and methyl jasmonate showed an ethylene peak at 13 DAH, when they acquired a reddish color.

It was possible to observe that the use of exogenous methyl jasmonate hormone in fruits with ethylene receptors blocked by 1-methylcyclopropene stimulated the ripening process when compared with those fruits treated only with 1-methylcyclopropene. This behavior induce that 1-methylcyclopropene is efficient to block ethylene receptors and consequently may avoid the interaction of ethylene with others phytohormones related to ripening processes such as the endogenous methyl jasmonate, occasioning the delay of fruit ripening. However, when doses of exogenous methyl jasmonate hormone was applied in these fruits an acceleration in ripening was observed by the accumulation of pigments and anticipation of ethylene peak from 21 to 13 DAH. In addition, the highest peak of ethylene emission was observed in MCP+MeJA group which may be related to stimulation of ethylene biosynthesis in climacteric fruits by methyl jasmonate hormone. From that, our results suggest that the exogenous methyl jasmonate can act independently of ethylene or the blockage of ethylene receptors were reversed after some period. Therefore, for the treatment with 1-methylciclopropene a synthesis of new receptors in tomato could be possible as related in several fruits [22,23]. This behavior may be responsible for the increase of ethylene production after some period, as it was observed after 10 DAH.

3.2 Primary metabolite profiling affected by postharvest hormonal treatment

Primary metabolites are major components of fruit quality and related metabolisms are considered crucial for plant growth. For this fact, more advances in its comprehension can facilitate the finding of future strategies for manipulation of fruit metabolism [24]. In this work, a total of 46 primary metabolites were identified by metabolomics analysis: 10 sugars (glucose, fructose, sucrose, allose, gulose, glucaric acid, myo-inositol, mannose, ribose, and arabinofuranse); 9 organic acids (oxaloacetic, citric, succinic, aconitic, malic, citraconic, fumaric, propanoic, and butanoic acids); 12 amino acids (proline, serine, valine, threonine, aspartic acid, glutamic acid, glutamine, γ-aminobutyric acid (GABA), asparagine, tryptophan, phenylalanine, and tyrosine); 12 saturated fatty acids (capric, lauric, myristic, palmitic, stearic, eicosanoic, docosanoic, tricosanoic, lignoceric, hyenic, cerotic, and montanic acids); and 3 unsaturated fatty acids (oleic, linoleic, and linolenic acids) at 4, 10 and 21 DAH (Table 1). Also, a global overview of the metabolic changes occurring in tomato during ripening was performed to evaluate significant differences among accumulated metabolites in treated fruits compared with control group (Figure 2).

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Table 1. Primary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both 1-methylcyclopropene and methyl jasmonate (MCP+ MeJA) treatments at 04, 10 and 21 days after harvest (DAH) detected by gas chromatography-mass spectrometry (GC-MS).

Metabolite	04 DAH			10 DAH			21 DAH		
	CTRL	*MCP*	*MCP+MeJA*	*CTRL*	*MCP*	*MCP+MeJA*	*CTRL*	*MCP*	*MCP+MeJA*
A) Sugars									
Glucose	1534,5±76,0^{d}	70,6±4,67^{g}	598,7±37,9^{f}	1977,6±11,4^{c}	156,5±7,5^{g}	2977,0±253,0^{b}	4352,0±281,0^{a}	189,4±15,2^{g}	1112,6±140,1^{e}
Fructose	27474,0±4039,0^{d}	3935,0±489,0^{e}	37418,0±5231,0^{c}	59266,0±6310,0^{b}	4858,0±544,0^{e}	26343,0±3352,0^{d}	101194,0±5662,0^{a}	25944,0±1592,0^{d}	24324,0±1808,0^{d}
Sucrose	38205,0±569,0^{c}	5105,0±559,0^{f}	20507,0±1161,0^{d}	54654,0±716,0^{b}	3661,0±356,0^{f}	11961,0±469,0^{e}	84839,0±4545,0^{a}	10885,0±358,0^{e}	11248,0±284,0^{e}
Allose	1098,6±44,2^{c}	172,6±12,6^{ef}	115,1±4,78^{f}	1563,4±21,5^{b}	630,6±39,0^{d}	687,0±56,9^{d}	3309,0±380,0^{a}	469,1±49,6^{de}	584,1±27,8^{d}
Gulose	221,5±9,78^{d}	196,9±15,1^{d}	229,8±10,9^{d}	790,4±38,3^{b}	92,6±4,47^{e}	228,8±10,4^{d}	1017,0±60,4^{a}	183,5±17,6^{d}	404,0±12,9^{c}
Glucaric acid	42,2±1,61^{d}	21,9±2,08^{e}	23,1±0,44^{e}	72,0±1,30^{c}	93,4±6,25^{b}	126,7±8,50^{a}	124,6±8,69^{a}	15,1±1,33^{e}	21,2±0,83^{e}
Myo-inositol	77,9±2,91^{e}	33,1±3,20^{f}	91,3±4,53^{e}	169,6±2,04^{d}	178,5±8,05^{d}	156,6±4,09^{d}	340,1±20,6^{c}	412,4±23,9^{b}	481,0±9,67^{a}
Mannose	42,2±3,10^{d}	3,37±0,22^{f}	6,00±0,26^{f}	100,0±2,30^{b}	69,5±3,46^{c}	148,3±4,68^{a}	142,4±11,04^{a}	28,9±2,42^{e}	66,9±9,67c
Ribose	174,6±7,42^{c}	5,89±0,40^{g}	14,35±0,57^{g}	249,4±3,91^{b}	26,2±1,29^{g}	128,5±3,75^{d}	386,4±27^{a}	60,7±6,44^{f}	89,8±3,46^{f}
Arabinofuranose	15,1±0,74^{c}	2,12±0,28^{e}	4,74±0,13^{e}	25,5±0,84^{b}	9,33±0,62^{d}	8,06±0,54^{d}	45,2±3,28^{a}	17,17±1,44^{c}	17,61±1,61^{c}
Total	68885,0±4082,0^{c}	9546,0±771,0^{e}	59009,0±5831,0^{c}	118868,0±5993,0^{b}	9775,0±904,0^{e}	42764,0±3869,0^{d}	195750,0±9973,0^{a}	38206,0±1874,0^{d}	38348,0±2196,0^{d}
B) Organic acids									
Oxaloacetic acid	573,3±24,3^{e}	173,3±18,6^{f}	214,5±6,23^{f}	2380,4±56,5^{a}	1234,0±59,1^{c}	1482,7±55,4^{b}	1241,9±80,7^{c}	703,7±72,1^{d}	823,0±24,2^{d}
Citric acid	6517,0±413,0^{c}	765,4±85,1^{e}	998,9±62,0^{e}	7878,0±457,0^{c}	889,9±79,5^{e}	4117,0±591,0^{d}	18901,0±1208,0^{a}	15494,0±1393,0^{b}	15948,0±747,0^{b}
Succinic acid	2646,0±360,0^{cd}	642,8±49,6^{f}	1909,3±81,4^{de}	12894,0±485,0^{a}	2826,0±650,0^{c}	3587,5±102,0^{b}	2862,0±254,0^{bc}	1851,1±135,1^{e}	2372,9±71,7^{cde}
Aconitic acid	61,1±2,90^{c}	2,91±0,31^{f}	52,9±2,63^{d}	83,1±0,86^{b}	4,22±0,63^{f}	51,0±1,06^{d}	101,4±5,70^{a}	18,2±1,57^{e}	67,1±2,84^{c}
Malic acid	2537,5±101,3^{d}	118,7±5,34^{f}	156,1±6,21^{f}	6653,7±174,0^{c}	1208,0±109,4^{ef}	2076,1±46,3^{de}	16800,0±1014,0^{a}	13024,0±1137,0^{b}	12285,0±385,0^{b}
Citraconic acid	17,3±0,74^{c}	4,15±0,34^{d}	3,73±0,24^{d}	104,5±12,4^{a}	3,87±0,18^{d}	2,74±0,30^{d}	101,9±6,07^{a}	20,9±1,86^{c}	49,0±2,18^{b}
Fumaric acid	167,9±6,66^{c}	61,0±5,29^{fg}	73,4±2,73^{f}	181,7±1,17^{b}	47,7±1,99^{h}	99,0±2,98^{e}	237,8±10,3^{a}	55,8±5,18^{gh}	128,6±5,42^{d}
Propanoic acid	111,7±6,77^{c}	14,6±2,06^{e}	19,0±1,17^{de}	145,6±1,5^{c}	15,4±0,76^{e}	15,4±0,50^{e}	458,6±79,1^{b}	90,7±5,02^{cd}	1318,0±51,3^{a}
Butanoic acid	284,5±18,9^{cd}	221,3±19,7^{d}	314,9±8,60^{cd}	393,4±10,9^{c}	179,5±8,62^{d}	274,4±5,89^{cd}	641,8±96,9^{b}	412,7±22,5^{c}	2404,7±175,3^{a}

Continued Table 1

Metabolite	04 DAH			10 DAH			21 DAH		
	CTRL	*MCP*	*MCP+MeJA*	*CTRL*	*MCP*	*MCP+MeJA*	*CTRL*	*MCP*	*MCP+MeJA*

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Total	12917,0±804,0^{d}	2003,4±173,2^{f}	3743,0±130,7^{ef}	30715,0±907,0^{c}	6409,0±787,0^{e}	11706,0±691,0^{d}	41346,0±2535,0^{a}	31671,0±2748,0^{c}	35397,0±1239,0^{b}
C) Amino acids									
Proline	501,2±14,8^{d}	798,4±71,8^{bcd}	992,4±55,3^{bc}	1167,2±26,8^{b}	693,5±40,9^{cd}	470,9±38,8^{d}	2863,8±483,0^{a}	2546,8±166,2^{a}	2452,7±44,8^{a}
Serine	78,0±5,68^{b}	24,8±1,38^{e}	58,5±4,32^{c}	93,6±2,26^{a}	27,7±1,98^{e}	24,8±1,02^{e}	49,0±1,56^{d}	16,1±1,95^{f}	26,9±1,35^{e}
Valine	6,22±0,18^{e}	1,89±0,18^{f}	2,24±0,04^{f}	17,6±0,24^{a}	17,0±0,76^{a}	14,9±0,52^{b}	7,93±0,40^{d}	6,60±0,89^{e}	9,10±0,30^{c}
Threonine	6,01±0,45^{e}	4,29±0,59^{e}	6,42±0,11^{de}	25,9±0,44^{b}	9,88±0,48^{d}	14,9±0,54^{c}	25,2±0,85^{b}	26,4±2,71^{b}	50,5±3,51^{a}
Aspartic acid	1523,2±54,2^{b}	54,7±4,83^{e}	141,7±3,94^{e}	2166,8±58,7^{a}	823,2±42,5^{d}	1163,1±43,0^{c}	1540,8±161,0^{b}	1148,6±97,4^{c}	1116,9±43,0^{c}
Glutamic acid	1744,3±75,6^{d}	215,3±17,3^{e}	228,7±8,32^{e}	4906,7±42,6^{a}	1614,2±71,8^{d}	1530,1±50,3^{d}	3957,0±424,0^{b}	2729,0±208,0^{c}	2551,6±68,0^{c}
Glutamine	185,1±7,71^{c}	77,8±5,64^{e}	172,9±4,83^{c}	519,3±9,24^{a}	99,9±5,26^{de}	148,3±4,71^{cd}	475,8±38,7^{a}	351,8±38,2^{b}	314,9±24,4^{b}
GABA	207,6±17,2^{bc}	148,4±13,2^{de}	173,9±4,47^{cd}	1060,8±45,9^{a}	104,3±4,63^{ef}	76,9±4,36^{f}	216,9±20,6^{bc}	221,9±23,6^{bc}	242,5±12,0^{b}
Asparagine	139,7±6,22^{c}	19,8±1,72^{e}	22,6±0,52^{e}	260,2±6,88^{a}	117,6±5,58^{d}	142,8±4,84^{c}	235,6±11,7^{b}	134,2±10,9^{cd}	148,5±11,1^{c}
Tryptophan	173,7±7,10^{b}	21,9±1,96^{d}	22,8±0,87^{d}	321,4±8,65^{a}	154,6±7,93^{c}	154,2±4,93^{c}	27,1±0,94^{d}	25,0±1,78^{d}	31,9±2,85^{d}
Phenylalanine	7,64±0,47^{e}	76,2±9,84^{a}	69,1±2,14^{a}	26,4±1,32^{d}	5,90±0,30^{e}	8,33±0,38^{e}	50,9±2,67^{b}	36,3±4,96^{c}	36,4±1,61^{c}
Tyrosine	21,1±0,77^{e}	47,8±3,61^{c}	71,0±3,93^{a}	27,8±2,37^{de}	35,7±2,0^{d}	27,7±1,08^{de}	62,3±3,70^{b}	51,9±5,01^{c}	46,4±4,33^{c}
Total	4593,8±170,8^{d}	1491,4±125,1^{f}	1962,3±70,7^{f}	10593,8±94,5^{a}	3703,5±149,7^{e}	3777,0±148,3^{de}	9512,0±940,0^{b}	7294,0±482,0^{c}	35397,0±1239,0^{b}
D) Saturated fatty acids									
Capric acid	61,9±1,90^{a}	26,6±0,62^{c}	52,2±0,71^{b}	15,0±0,41^{d}	1,48±0,08^{g}	2,27±0,01^{g}	6,77±0,03^{e}	4,05±0,11^{f}	5,76±0,13^{ef}
Lauric acid	40,5±2,10^{a}	18,9±0,08^{c}	22,4±0,35^{b}	19,7±1,16^{c}	1,40±0,07^{e}	2,14±0,04^{e}	6,81±0,03^{d}	5,56±0,11^{d}	5,91±0,15^{d}
Myristic acid	19,0±0,83^{a}	0,76±0,03^{f}	2,08±0,02^{e}	15,0±0,50^{b}	1,25±0,05^{f}	2,56±0,03^{e}	6,13±0,07^{c}	4,89±0,12^{d}	5,94±0,13^{c}
Palmitic acid	436,7±10,4^{a}	148,7±5,32^{e}	285,9±23,0^{d}	413,1±10,9^{ab}	23,9±0,91^{f}	36,2±0,31^{f}	159,6±1,41^{e}	349,8±6,23^{c}	400,5±9,30^{b}
Stearic acid	16,5±0,49^{a}	9,20±0,52^{ef}	8,60±0,16^{f}	14,6±0,26^{b}	1,01±0,05^{h}	13,2±0,07^{c}	9,94±0,18^{d}	7,61±0,23^{g}	9,34±0,07^{de}
Eicosanoic acid	93,9±1,59^{b}	84,1±6,01^{c}	170,9±2,83^{a}	10,19±0,31^{d}	5,76±0,25^{de}	8,73±0,17^{d}	7,16±0,17^{d}	7,10±0,11^{d}	0,74±0,02^{de}
Docosanoic acid	43,7±1,04^{b}	23,6±2,36^{c}	53,8±1,60^{a}	41,3±1,09^{b}	6,72±0,29^{f}	10,9±0,13^{e}	16,0±0,14^{d}	8,22±0,19^{f}	9,24±0,42^{ef}
Tricosanoic acid	27,6±1,15^{b}	22,7±1,28^{c}	45,2±0,31^{a}	20,3±0,28^{d}	5,50±0,26^{g}	8,83±0,18^{e}	7,08±0,17^{f}	6,62±0,13^{fg}	7,68±0,27^{ef}
Lignoceric acid	22,1±1,82^{c}	163,6±5,06^{b}	618,9±23,1^{a}	13,9±0,19^{c}	5,27±0,21^{c}	11,1±0,48^{c}	7,17±0,23^{c}	6,84±0,12^{c}	7,79±0,39^{c}

Continued Table 1

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***MCP***	***MCP+MeJA***	***CTRL***	***MCP***	***MCP+MeJA***	***CTRL***	***MCP***	***MCP+MeJA***
Hyenic acid	32,5±1,05^{b}	25,8±0,23^{c}	37,2±1,73^{a}	8,82±0,21^{d}	3,27±0,13^{f}	6,92±0,02^{e}	6,58±0,08^{e}	5,66±0,10^{e}	7,28±0,15^{de}

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Cerotic acid	10,0±0,70^{b}	7,86±0,38^{cd}	26,9±1,64^{a}	10,2±0,31^{b}	5,76±0,25^{e}	8,73±0,17^{bc}	7,29±0,16^{cd}	7,18±0,11^{de}	7,53±0,28^{cd}
Montanic acid	34,4±1,33^{a}	15,2±0,40^{c}	17,7±0,63^{b}	9,49±0,22^{d}	5,37±0,23^{f}	7,39±0,08^{e}	9,85±0,16^{d}	6,11±0,12^{f}	9,13±0,15^{d}
Total	838,8±20,6^{b}	547,0±6,96^{d}	1341,7±15,0^{a}	591,6±14,5^{c}	66,7±2,68^{i}	119,0±0,51^{h}	250,4±2,03^{g}	419,7±7,50^{f}	476,8±11,0^{e}
E) Unsaturated fatty acids									
Oleic acid	610,5±26,0^{b}	278,4±5,43^{d}	866,7±20,9^{a}	339,4±7,63^{c}	19,9±0,92^{g}	44,8±0,42^{g}	213,0±7,05^{e}	103,6±10,5^{f}	340,2±6,53^{c}
Linoleic acid	1079,5±56,5^{c}	8,77±0,51^{g}	46,9±0,31^{g}	749,6±21,4^{d}	285,0±14,9^{f}	588,5±16,7^{e}	593,1±5,06^{e}	5065,0±93,4^{b}	5880,9±144,9^{b}
α-Linolenic acid	94,9±4,36^{c}	354,6±6,55^{a}	292,1±1,54^{b}	24,0±0,33^{ef}	11,3±0,53^{g}	33,2±0,14^{d}	17,3±2,32^{fg}	14,1±0,44^{g}	25,3±1,10^{e}
Total	1784,9±78,7^{c}	641,8±10,3^{f}	1205,7±22,1^{d}	1113,1±29,0^{d}	316,2±15,8^{g}	666,5±16,4^{f}	823,4±11,1^{e}	5183,7±89,5^{b}	6246,4±147,8^{a}

Values were presented as normalized area by ribitol or n-tridecane (internal polar and non-polar standards, respectively). CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation, n = 4). GABA, γ-aminobutyric acid.

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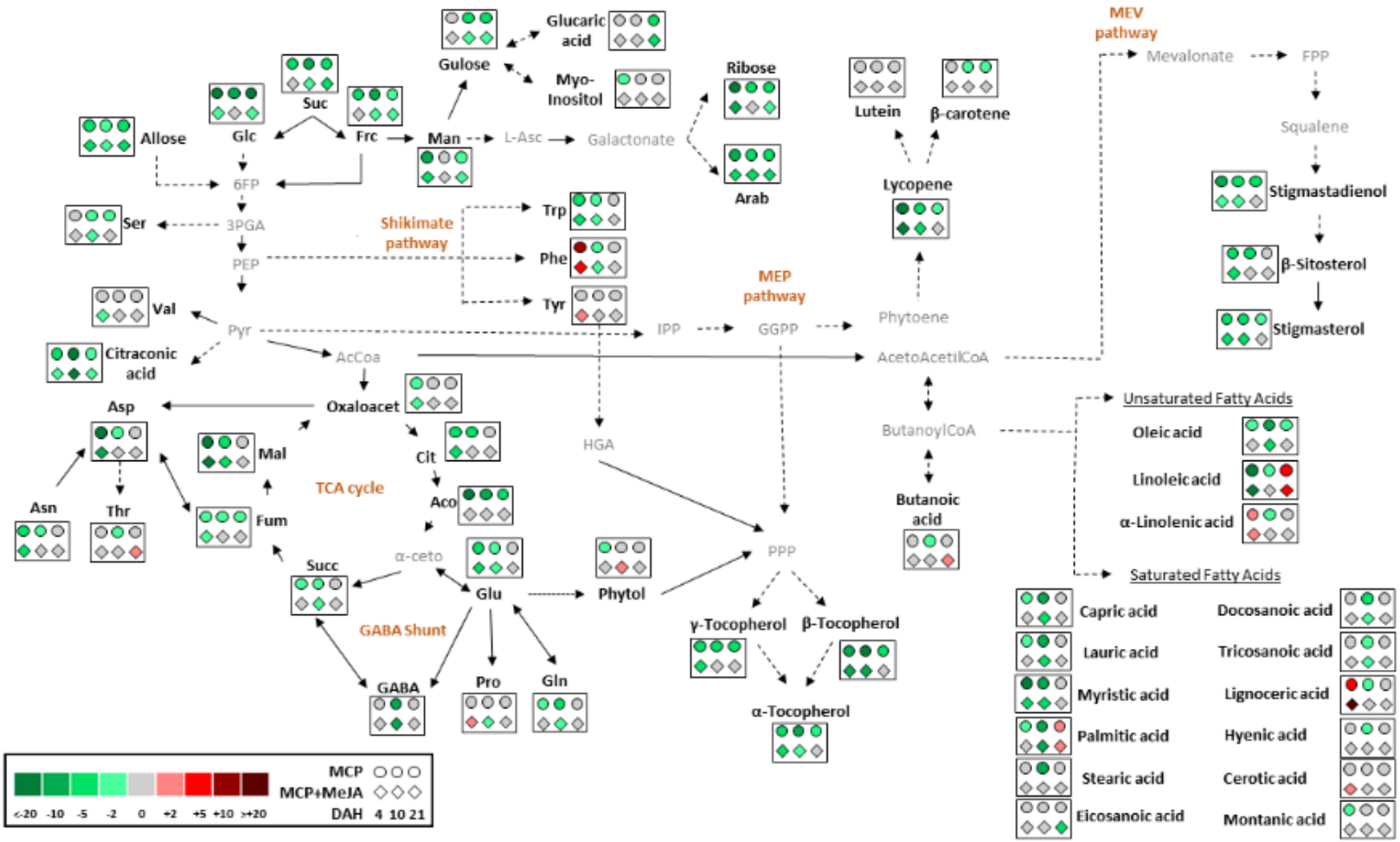

Figure 2. Global overview of metabolic changes occurring in tomato (*Solanum lycopersicum* L. cv. Grape) treated with 1-methylcyclopropene (MCP) and both hormones 1-methylcyclopropene and methyl jasmonate (MCP+MeJA) compared to control group (CTRL). Data were normalized to CTRL. Metabolites showing up- or down-regulation in each treatment higher 2.0 fold than CTRL are showed. Color scale is used to display the different amount of metabolite in terms of fold-change relative to the level in the appropriate control. Suc, sucrose; Glc, glucose; Frc, fructose; Man, Mannose; L-Asc, L-ascorbic acid; Arab, Arabinofuranose; 6FP, fructose-6-phosphate; 3-GPA, glyceraldehyde-3-phosphate; Ser, serine; PEP, phosphoenolpyruvate; Trp, tryptophan; Phe, phenylalanine; Tyr, Tyrosine; HGA, homogentisic acid; Pyr, pyruvic acid; IPP, isopentenyl pyrophosphate; GGPP, geranylgeranyl; PPP, phytyl pyrophosphate; Val, valine; AcCoA, acetyl-CoA;

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Oxaloacet, oxaloacetic acid; Cit, citric acid; Aco, aconitic acid; α-ceto, α-cetoglutaric acid; Succ, succinic acid; Fum, fumaric acid; Mal, malic acid; Glu, glutamic acid; GABA, γ-aminobutyric acid; Gln, glutamine; Pro, proline; Arg, arginine; Asp, aspartic acid; Thr, threonine; Asn, asparagine; FPP, farnesyl pyrophosphate.

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A PCA was performed on primary metabolites at 4th, 10th and 21th ripening stages and confirmed the high reproducibility among the four biological replicates and groups analyzed. Also, a clearly separation of CTRL group and both treated groups was evidenced for the primary metabolites in the PCA-score. Heatmap analysis was used to analyze the differences between treated and no-treated groups regarding the metabolites changes at each day after harvest analyzed (Figure 3, 4 and 5).

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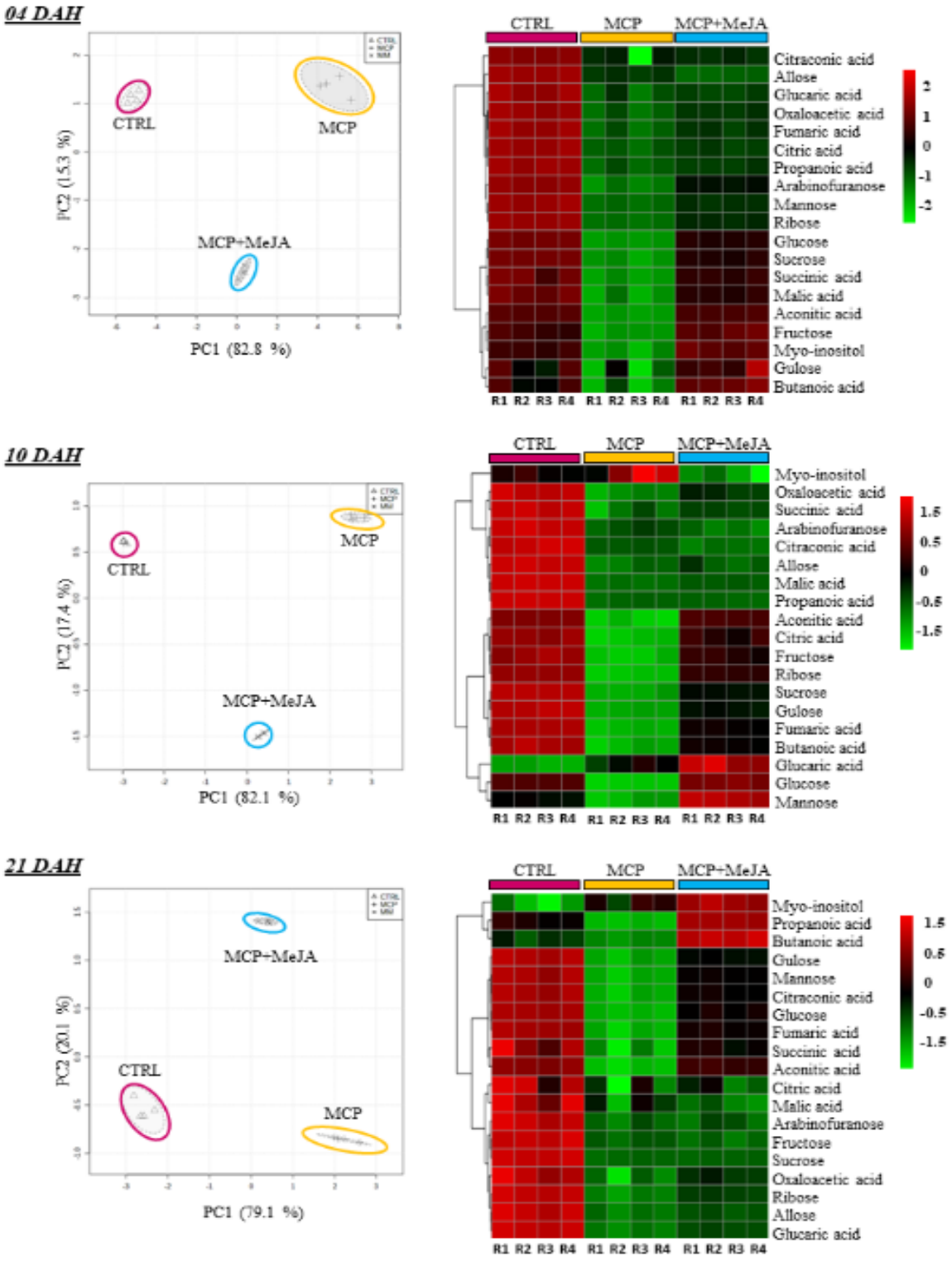

Figure 3. Relative contents of sugars and organic acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both 1-methylcyclopropene and methyl jasmonate (MCP+MeJA) treatments compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

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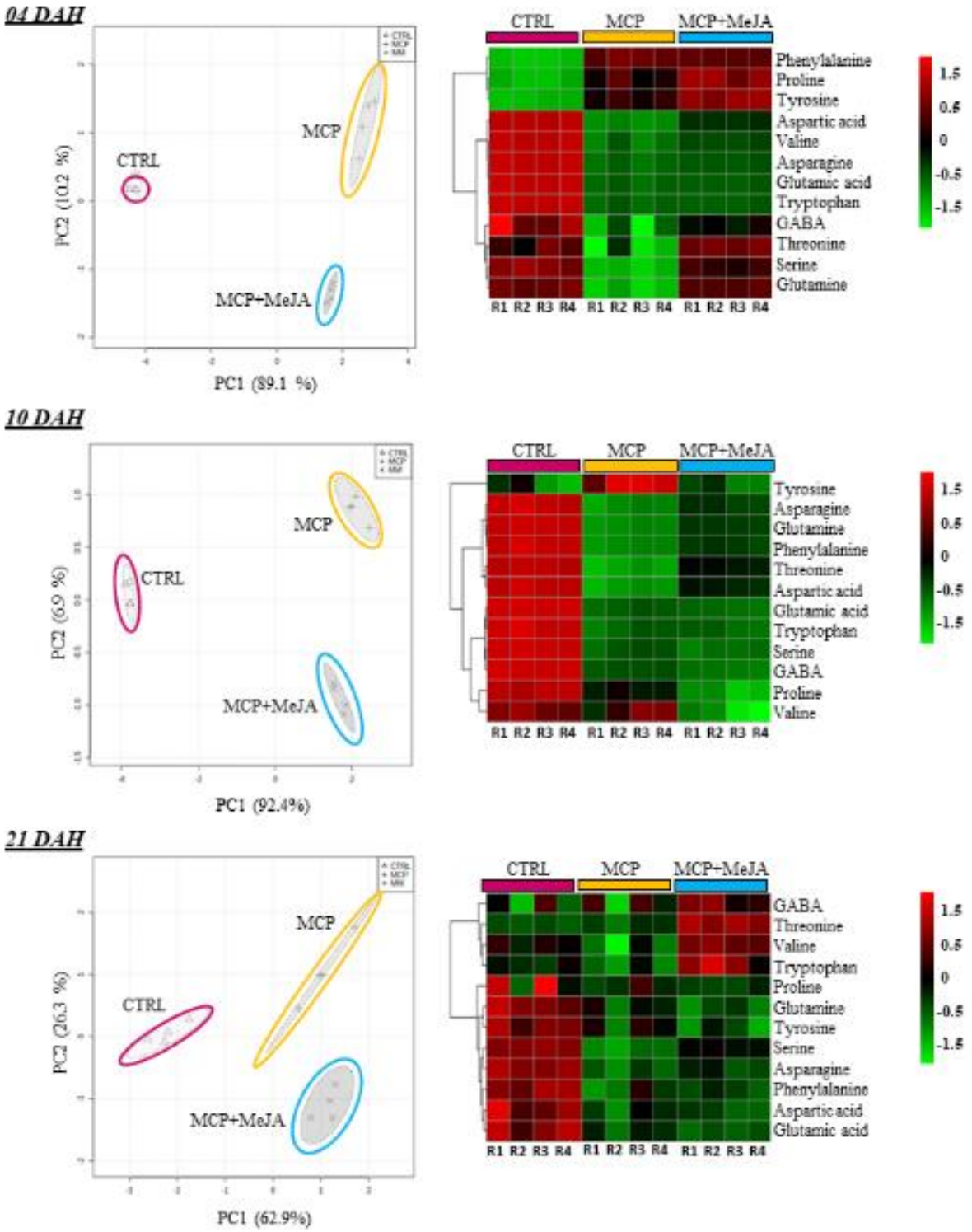

Figure 4. Relative contents of amino acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones 1-methylcyclopropene and methyl jasmonate (MCP+MeJA) treatments compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

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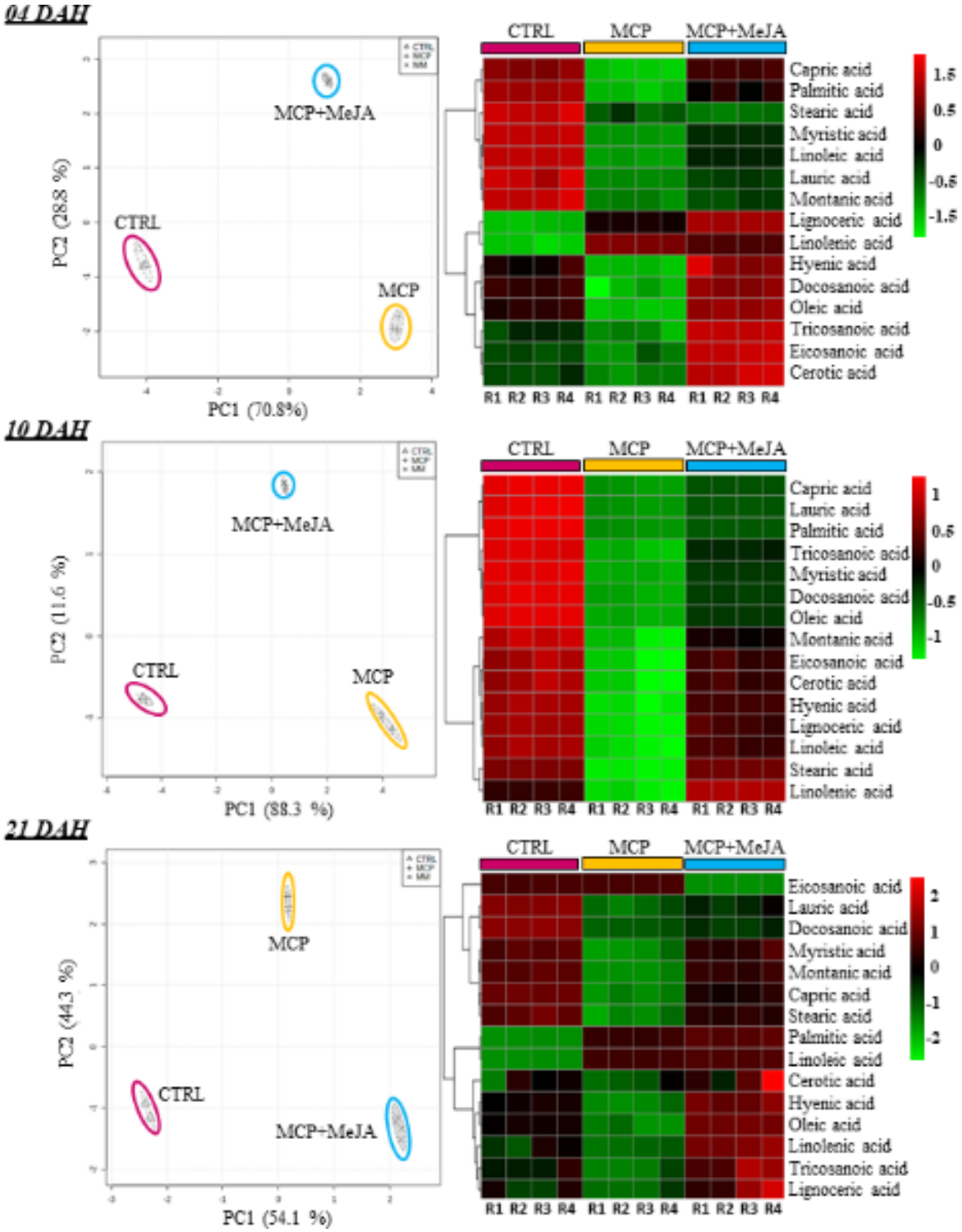

Figure 5. Relative contents of fatty acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones 1-methylcyclopropene and methyl jasmonate (MCP+MeJA) treatments compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

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Definitely, treatment with 1-methylcyclopropene impacted sugar and organic acids, inhibiting their production during ripening. Fruits treated only with 1-methylcyclopropene were most affected, showing a greater delay in accumulate sugars and organic acids than those fruits treated with both 1-methylcyclopropene and methyl jasmonate (Figure 2). For instance, glucose showed a significantly reduction of 22, 13 and 23 fold at 4, 10 and 21 DAH, respectively, in MCP when compared with CTRL. Mannose, ribose, malic and aconitic acids exhibited a decrease in their levels of 14, 30, 21 and 20 fold at 4 DAH, whereas fructose, sucrose and citraconic acid showed 12, 15 and 27 fold lower levels at 10 DAH when compared with CTRL (Table 1, Figure 2).

Exceptionally, levels of glucose, glucaric acid and mannose showed an increase at 10 DAH in MCP+MeJA when compared to CTRL. Similar behavior was observed by myo-inositol, propanoic and butanoic acids at 21 DAH (Table 1, Figure 3). As observed by ethylene emission, the minor impact on the production sugars and organic acids observed by MCP+MeJA may suggest that methyl jasmonate play an important role in ripening process, which may act independently of endogenous ethylene, or a stimulation of the synthesis of new receptors, or the blockage of ethylene receptors were reversed after some period.

Amino acids profiling were also affected by the action of 1-methylcyclopropene. A inhibition in the production of amino acids during ripening were observed in both MPC and MCP+MeJA when compared with control (Figure 2 and 4). The most affected amino acids were aspartic acid at 4 DAH and GABA at 10 DAH, showing a reduction in their levels of 28 and 10 fold in MPC, respectively, while MCP+MeJA showed 11 and 14 fold decreased, respectively, when compared with CTRL. In contrast, tyrosine and phenylalanine showed levels 2 and 9 fold higher in MCP and MCP+MeJA at 4 DAH when compared with CTRL (Table 1, Figure 2). Phenylalanine and tyrosine are important aromatic amino acids, which participate of shikimate pathway and are responsible for aroma development of fruit. The total amino acids level was represented mostly by proline, glutamic and aspartic acids, which are important to fruit quality (Table 1).

In addition, fatty acids profiling were also affected by the post-harvest treatments. The action of 1-methylciclopropene showed a greater impact on fatty acids such as oleic, capric, lauric, palmitic, stearic and myristic acids at 10 DAH, decreasing their levels 17, 10, 14, 17, 14 and 12 fold in MPC group, respectively, and 7, 6, 9, 11, 1, 7 fold in MCP+MeJA, respectively, when compared with CTRL. MCP+MeJA group also showed a reduction in fatty acids levels, but they were lesser impacted when compared with MPC group. However, the most impacted was the linoleic and myristic acids at 4 DAH with a reduction of 119 and 26 fold in MCP, respectively, and 23 and 9 in MCP+MeJA, respectively, when compared with CTRL (Table 1, Figure 2).

In contrast, an increase in the levels of some fatty acids was also detected as well as in lignoceric, cerotic, α-linolenic acids at 4 DAH, and palmitic and linoleic acids at 21 DAH by MCP and MCP+MeJA groups (Figure 2 and 5). In MCP group was detected an increase in the levels of lignoceric and α-linolenic acids at 4 DAH by 7 and 4 fold, respectively, while in MCP+MeJA the increase was 28 and 3 fold, respectively. Moreover, palmitic and linoleic acids was increased by 2 and 8 fold, respectively, in MCP, and 3 and 10, respectively, in MCP+MeJA at 21 DAH (Table 1). Interesting, MCP+MeJA group was lesser impacted when reductions were observed, and greater impacted when increases were observed comparing with MCP. This behavior may induce that methyl jasmonate can act as stimulator in the production of fatty acids. Palmitic and eicosanoic acids contributed essentially with the total of saturated fatty acids level, whereas oleic and linoleic acids with the total of unsaturated fatty acids level.

3.3 Secondary metabolite profiling affected by postharvest hormonal treatment

The secondary metabolites identified in tomato fruits at 4, 10 and 21 DAH were: lycopene, β-carotene, and lutein by HPLC analysis; and α-tocopherol, β-tocopherol, γ-tocopherol, phytol, β-sitosterol, estigmasterol, and estigmastadienol by GC-MS analysis.

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Lycopene was the most affected by the action of 1-methylciclopropene, reducing its level not only in MCP, but also in MCP+MeJA by 29 and 25 fold, respectively, at 4 DAH, while at 10 DAH the reduction was 8 and 6 fold, respectively, when compared with CTRL. At 21 DAH, lycopene suffer a reduction of 2.8 in MCP. The impact lower than 2 fold in the level of carotenoids of the both treatments compared with CTRL can be observed also in the Figure 2. A reduction lower than 2-fold was observed in β-carotene and lutein at 4, 10 and 21 DAH, exceptionally for β-carotene which reduced 2.4 fold in MCP at 21 DAH (Figure 6A, Table S1). In contrast, an increase in carotenoids levels were detected at 21 DAH in MCP+MeJA. Lycopene and β-carotene showed an increase of 10 %, and lutein of 20% when compared with CTRL (Figure 6A, Table S1). Total carotenoids level was represented mainly by lycopene.

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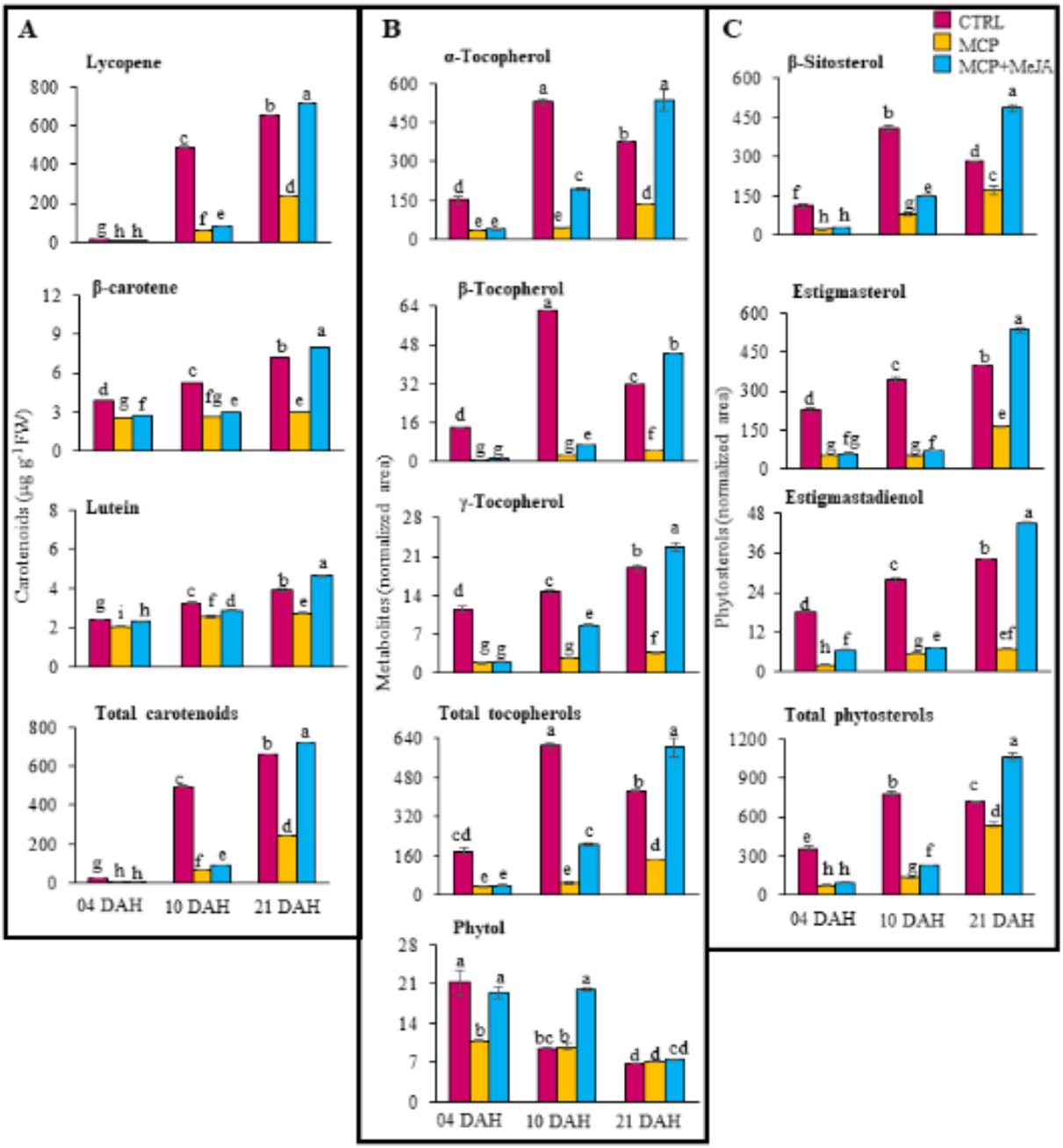

Figure 6. Secondary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones 1-methylcyclopropene and methyl jasmonate (MCP+MeJA) treatments compared to the control group (CTRL) at 04, 10 and 21 days after harvest (DAH). Contents of carotenoids (A), normalized area of tocopherols and phytol (B), and phytosterols (C). Values are means ± SE of four biological replicates of 10 fruits each. Different letters indicate statistically significant differences ($p < 0.05$).

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Tocopherol profiling showed a similar behavior that carotenoids during ripening, with decreasing in its levels in both treatments groups at 4 and 10 DAH (Figure 2), and at 21 DAH presented a decrease in MPC group and an increase in MCP+MeJA of tocopherols when compared with CTRL. Levels of α-tocopherol showed a reduction in MCP and MCP+MeJA of 5 and 4 fold, respectively, at 4 DAH, while at 10 DAH decreased 12 and 3 fold, respectively. β-tocopherol levels suffer a reduction of 14 and 12 fold at 4 DAH, and 23 and 9 fold at 10 DAH in MCP and MCP+MeJA, respectively. In addition, α-tocopherol was decreased by 6 fold at 4 and 10 DAH in both treatments groups, exceptionally for the MCP+MeJA at 10 DAH which decreased 1.7 fold when compared with CTRL (Figure 6B, Table S2).

In contrast, at 21 DAH tocopherol profiling was lesser affected by 1-methylcyclopropene, and impacted positively by the concomitant treatment of 1-methylcyclopronene and methyl jasmonate, showing an improved of 40 % in the levels of α-tocopherol and β-tocopherol and 21 % in the levels of γ-tocopherol when compared with CTRL (Figure 6B, Table S2). Total tocopherols level was characterized mainly by the content of α-tocopherol. An acyclic diterpenoid identified was phytol, which presented a reduction of 2 fold in MPC at 4 DAH and increase of 2 fold in MCP+MeJA at 10 DAH (Figure 6B, Table S2). The impact of these treatments at 4 and 10 DAH can be observed also in the Figure 2.

Phytosterols were also affected by 1-methylcyclopropene, showing reductions in β-sitosterol levels of 5 fold in MCP at 4 and 10 DAH, and 3 fold in MCP+MeJA at 4 and 10 DAH, comparing with CTRL. Estigmasterol exhibited reduction in MCP of 4 and 7 fold at 4 and 10 DAH, respectively, while MCP+MeJA showed a decrease of 3 and 5 fold at 4 and 10 DAH, respectively. Estigmastadienol was the most affected by 1-methylcyclopropene, decreasing 9 fold at 4 DAH (Figure 6C, Table S2). β-sitosterol and estigmasterol was the major source of total phytosterols level. Besides, the down-regulation higher than 2 fold compared with CTRL can be observed by the phytosterols in the Figure 2. Divergently of behavior of phytosterols profiling at 4 and 10 DAH, β-sitosterol, estigmasterol and estigmastadienol showed an enhancement in their levels of 42, 34 and 32 %, respectively, in fruits treated with both 1-methylcyclopropene and methyl jasmonate at 21 DAH (Figure 6C, Table S2).

3.4 Lipid metabolism affected by the postharvest jasmonate treatment

The metabolite profiling of tomato fruits treated with only 1-methylcyclopropene and with both 1-methylcyclopropene and methyl jasmonate showed a significant impact to the fruit quality and, consequently, to the ripening process. Although the profiles of sugars, organic acids and amino acids were affected by the treatment with jasmonate, the most remarkable deference observed was found in the metabolism of lipids.

Oleic, capric, lauric, palmitic, stearic and myristic acids showed a reduction of 17 fold when treated with only 1-methylcyclopropene and up to 11 times when treated with both methyl jasmonate and 1-methylcyclopropene, comparing with the no treated fruits at 10 DAH. Important to highlight the drastic decrease in the levels of linoleic and myristic acids at 4 DAH in both treatments (Table 1, Figure 2). However, an interesting increase in the levels of lignoceric, cerotic, α-linolenic acids at 4 DAH, and palmitic and linoleic acids at 21 DAH in both treatments was detected (Figure 2 and 5).

At 4 and 10 DAH, carotenoids, tocopherols and phytosterols suffered important reductions in their levels. Although, a notable accumulation in their levels was detected at 21 DAH (Figure 6, Table S1 and S2).

Fruits treated with methyl jasmonate had a positive impact on the accumulation of metabolites, mainly in the non-polar metabolites such as fatty acids, carotenoids, tocopherols and phytosterols. The addition of exogenous methyl jasmonate to the fruit, with ethylene receptors blocked, showed that methyl jasmonate can act independently of endogenous ethylene or suggests that the blocking of ethylene receptors was reversed after 10 DAH or new ethylene receptors were synthesized. Postharvest treatment with jasmonate showed that it is possible to obtain an improvement in the fruit quality with prolonged shelf life.

Supplementary Materials: Table S1: Carotenoids contents (μg.g^{-1} FW) in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both 1-methylcyclopropene and methyl jasmonate (MCP+ MeJA) treatments at 04, 10 and 21 days after harvest (DAH) detected by high performance liquid chromatography (HPLC); **Table S2:** Tocopherols, phytol and phytosterols in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both 1-methylcyclopropene and methyl jasmonate (MCP+ MeJA) treatments at 04, 10 and 21 days after harvest (DAH) detected by gas chromatography-mass spectrometry (GC-MS).

Author Contributions: Investigation, Writing and Original draft preparation: S.L.R.M.; Writing - Review & Editing: I.L.M., and E.C.T.; Methodology, Software: G.B.P; Data curation: I.L.M.; Supervision: E.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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