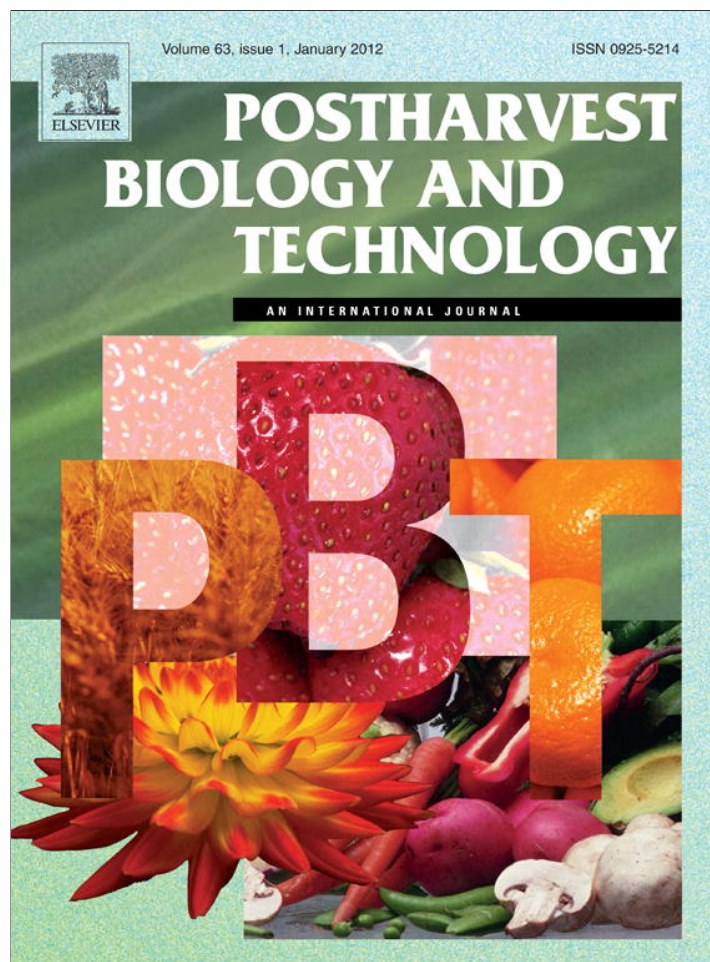


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# Postharvest Biology and Technology

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## Metabolite content of harvested Micro-Tom tomato (*Solanum lycopersicum* L.) fruit is altered by chilling and protective heat-shock treatments as shown by GC–MS metabolic profiling

Kietsuda Luengwilai<sup>1</sup>, Mikal Saltveit, Diane M. Beckles\*

Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA

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## ABSTRACT

The primary aim of this study was to identify metabolites associated with chilling tolerance that was engendered by a heat-shock treatment of tomato fruit pericarp (*Solanum lycopersicum* L. cv. Micro-Tom). Harvested mature-green fruit were immersed in 20 or 40 °C water for 7 min ('Heat-Shock') and then stored at 2.5 °C for 0 or 14 d ('Chilled'). A reduction in chilling injury symptoms (i.e., slow or abnormal ripening, increased ion leakage, and increased respiration following chilling) was used to select this heat-shock treatment as optimal. Using GC–MS (Gas Chromatography–Mass Spectrometry) metabolite profiling, 363 analytes were detected in fruit pericarp of which 65 are identified metabolites. Principal Component Analysis of these data led to distinct groups among the samples based on their treatments; 'Chilled' and 'Chilled + Heat-Shocked' fruit were markedly different from each other, while the 'Non-Chilled Control' and 'Heat-Shocked' fruit were similar and grouped closer to the 'Chilled + Heat-Shocked' fruit. These results indicate that the heat treatment provided protection from chilling in part by altering levels of fruit metabolites. The levels of arabinose, fructose-6-phosphate, valine and shikimic acid appear to be associated with this heat-shock induced chilling tolerance since their levels were altered in the 'Chilled' samples ( $p < 0.05$ ), relative to the control and the heat-shocked protected fruit. We also describe the metabolites we identified that could be further studied as being indicative of incipient chilling injury in mature-green tomato fruit.

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### 1. Introduction

Chilling injury (CI) is a complex disorder that is detrimental to the quality of tropical and subtropical fruit and often leads to severe economic loss (Sevillano et al., 2009). Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops produced globally (Luengwilai and Beckles, 2009) and like most subtropical fruit is susceptible to CI. When fruit are exposed to low, non-freezing temperatures, a number of physiological and biochemical changes can occur, including a failure to ripen normally, surface indentations, discoloration, and increased rates of respiration and water loss (Morris, 1982; Saltveit and Morris, 1990). An early event of CI is the marked increase in membrane permeability, with increased leakage of ions from within cells (i.e., symplast) to inter-cellular (apoplastic) spaces within the tissue (Saltveit, 2005). While

symptoms can develop during chilling, most become pronounced after the chilled tissue is held at a non-chilling temperature (ca. 20 °C) for a few days (Saltveit and Morris, 1990).

Chilling injury in plants has been studied for over a century, but little is known about its molecular basis. Pre-conditioning at temperatures of ~40–50 °C before chilling mitigates the development of CI symptoms and that mitigation may be mediated by altered protein synthesis including the synthesis and accumulation of heat shock proteins (HSPs) (Ding et al., 2001; Lurie and Klein, 1991; Lurie et al., 1997; Saltveit, 2005). Low temperature stress causes changes in the accumulation of distinct metabolites in many plant species (Bohnert et al., 1995; Gusta et al., 1996; Guy, 1990; Hannah et al., 2006; Kaplan et al., 2004; Kishitani et al., 1994) and changes in the levels of a subset in response to pre-treatments may also contribute to induced chilling tolerance (Sevillano et al., 2009).

We do not know the metabolites that are altered in the early phases of CI in mature-green tomato fruit. Most CI research on mature-green fruit focuses on secondary events, i.e. when visual symptoms are manifest making it difficult to separate the initial 'cause' from the subsequent 'effect'. Some attempts to study the physiological and transcriptional processes initiated early in chilling stress in breaker and ripened tomato fruit have been made

\* Corresponding author at: Department of Plant Sciences MS3, University of California, One Shields Avenue, Davis, CA 95616, USA. Tel.: +1 530 754 4779.

E-mail address: [dbeckles@ucdavis.edu](mailto:dbeckles@ucdavis.edu) (D.M. Beckles).

<sup>1</sup> Current address: Horticulture Department, Kasetsart University, Kamphaengsaen Campus, Kamphaengsaen, Nakorn Pathom 73140, Thailand.

(Gomez et al., 2009; Weiss and Egea-Cortines, 2009). For example Gomez et al. (2009) took a targeted approach to examine the biochemical changes associated with postharvest chilling of tomato fruit cv. Micro-Tom. They assayed sugars (glucose, fructose and sucrose), organic acids (malic, citric, succinic and tartaric acid) and some carotenoids of breaker and red-ripe fruit and found changes in response to increasing incubation (after as little as 4 d) at low temperature (6 °C) mainly in breaker fruit (Gomez et al., 2009). This work was important in showing that Micro-Tom is a good model for CI studies and in pinpointing how specific compound were altered.

In contrast to targeted measurement of compounds, GC–MS and other hyphenated mass spectrometry technologies are powerful as they provide a view of a larger set of metabolites (Roessner and Beckles, 2009). They are proving to be an invaluable tool for understanding metabolic and physiological processes in tomatoes (Carrari et al., 2006; Moco et al., 2006, 2007; Schauer et al., 2006), and are also useful in identifying metabolite fingerprint associated with various postharvest disorders in a range of tissues (Nicolai et al., 2010; Pico et al., 2010; Rudell et al., 2008, 2009; Vikram et al., 2004, 2006). Adoption of these technologies coupled with a good description of fruit physiology may become indispensable for understanding postharvest biology and for developing diagnostic tests for fruit quality.

The aim of this work was to better understand metabolic alterations associated with early events in chilling injury in mature green tomato fruit. We had two specific goals. First, we wished to determine if it is possible to distinguish, based on GC–MS metabolite profiling, harvested tomato fruit that were subjected to chilling stress from those with a prior heat-shock treatments that increase chilling tolerance before symptoms manifest. Second we wished to identify the specific metabolites, which changed in response to these treatments. Examining the response of mature-green tomato fruit rather than in breaker fruit may offer further insight since (i) they are more susceptible to chilling injury and (ii) the complex metabolic changes associated with ripening at breaker would not confound the analysis. GC–MS analysis was done in the same pericarp fruit tissue samples that were used to characterize the effects of heat-shock treatments that induced chilling tolerance and reduced the rate of chilling-induced ion leakage (Luengwilai et al., 2011). There are clear benefits to monitoring changes in physiological and metabolic processes simultaneously during chilling rather than relying on visual symptoms that develop during subsequent storage at non-chilling temperatures. Metabolites associated with the earliest response of sensitive tissue to chilling injury may be identified. Such metabolites may be investigated as a marker(s) for breeding programs and in ascertaining whether sensitive crops have been exposed to injurious chilling temperatures on their way to market.

## 2. Materials and methods

### 2.1. Plant growth, fruit sampling and postharvest treatments

Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) plants were grown as previously described (Luengwilai et al., 2011). Five to ten mature-green fruit (i.e., fruit with softened locular tissue and seeds that could not be cut with a knife; Saltveit, 1991) were hand harvested from each plant. Uniform (4–6 g, fresh weight), non-damaged fruit were washed in commercial bleach (1:20 dilution of 5% (v/v) sodium hypochlorite) and air-dried under a laminar flow hood.

The experimental design consisted of six replicates of four treatments: a control treatment (*Control*: non-heat-shock, non-chilled), a heat-shock treatment (*HS*: heat-shocked, non-chilled), a chilled treatment (*Chilled*: non-heat-shock, chilled), and a heat-shock/chilled treatment (*HS + Chilled*: heat-shocked, chilled). Fruit

were immersed in water at 20 °C (control) or at 40 °C (heat-shock) for 7 min. The fruit were then air-dried for 30 min before being chilled at 2.5 °C for 14 d as previously described (Luengwilai et al., 2011).

### 2.2. Metabolite sample extraction and derivatisation

Frozen pericarp disks from the base of each fruit were ground in liquid nitrogen and 50 mg were extracted in 1500 µL of 100% (v/v) methanol. The extract was vortexed for 10 s, shaken for 15 min at 70 °C, centrifuged for 15 min at 3200 × g, and the supernatant was then transferred to new tube. The pellet was mixed vigorously with 500 µL of DI water, centrifuged for 15 min at 3200 × g and the supernatant was combined with the previous extract. Aliquots of 50 µL of the supernatants were dried in a Speed Vac concentrator (SVC 100, Savant instrument, Famingdale, NY). The derivatisation and GC–MS system was done as previously described (Fiehn et al., 2008). Analytes (363) were automatically detected using the Bin-Base algorithm (Fiehn et al., 2008), and 65 were unambiguously identified by comparing their retention times to that derived from mass spectral libraries. The identities of the remaining compounds (298) are unknown, but they appear consistently between samples and therefore may have a biological origin.

### 2.3. Data analyses

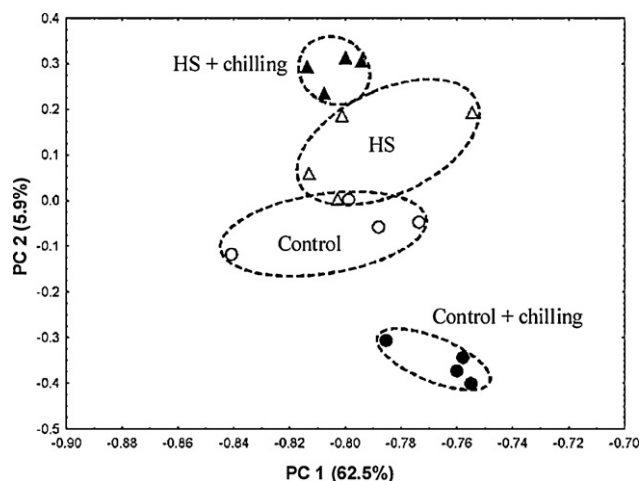
A one-way ANOVA and The Student's *t*-test at  $p < 0.05$  was used to detect significant changes due to chilling and heat-induced chilling tolerance. False discovery rates were calculated using Metaboanalyst (Xia et al., 2009). For all multivariate analyses the data were  $\log_{10}$  transformed prior to analysis.  $\log_{10}$  transformation provided the closest approximation to a normal distribution compared to other methods such as Pareto, Autoscaling and Range Scaling (van den Berg et al., 2006) contained within the Metaboanalyst program. Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) were performed using Statistica Dataminer (Statsoft, 2003). Both methods are designed to provide a general overview of the relationships among different samples (Stamova et al., 2009).

## 3. Results and discussion

### 3.1. Physiological response to chilling

Fruit-specific changes associated with the duration of chilling and chilling temperature are known to vary significantly from harvest to harvest and within and between experiments (Saltveit and Morris, 1990). Therefore we performed extensive physiological characterization of the chilling response (Luengwilai et al., 2011) and coupled that with a metabolomic study. As a result, molecular responses could be directly related to underlying biological processes in our experiments.

The rate of respiration of whole mature-green tomato fruit after chilling and the rate of chilling-induced ion leakage from excised mature-green pericarp tissue into an isotonic mannitol solution were both significantly reduced by heating the fruit to 40 °C for 7 min prior to chilling at 2.5 °C for 14 d. For 'Control' and 'HS' fruit – before chilling, the rate of ion leakage was  $3.3 \pm 1.1\%$  total conductivity per hour. After 14 d at 2.5 °C the rate of ion leakage from control fruit increased 3.3-fold to  $14 \pm 0.1\%$  total conductivity per hour, while the rate from 'HS' fruit only increased 2.4-fold to  $8.0 \pm 1.2\%$  total conductivity per hour (Luengwilai et al., 2011). As we wanted to relate the metabolic changes to physiological changes, GC–MS was performed on polar extracts of tomato fruit that were used for respiration and ion leakage measurement.



**Fig. 1.** Principal Component Analysis (PCA) of all 363 detected polar analytes measured by GC–MS in each tomato. Four biological replicates of each sample were plotted individually and the groups are as follows: fruit immersed in water at 20 °C or at 40 °C for 7 min are designated ('Control'; open circles) and ('HS'; open triangles) respectively. The fruit so treated were then air dried for 30 min before being chilled at 2.5 °C for 14 d. Chilled samples preceded by control conditions are designated ('Chilled'; solid circles) and by the protective HS treatment – ('HS + Chilled'; solid triangles). Each symbol on the plot represents data from 363 analytes in one biological replicate reduced to the first and second PC. Together, PC1 and PC2 captured 74% of the variance within the samples. Samples that have similar metabolite profiles will therefore group together.

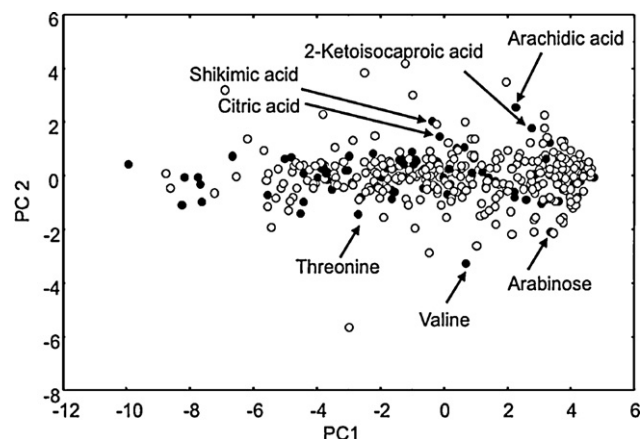
### 3.2. Multivariate analysis of GC–MS data

In order to examine the relationships among the groups of polar metabolites identified as a result of each of the contrasting treatments, Principal Component Analysis and Discriminant Function Analysis, including all 363 analytes detected, were performed (Fig. 1).

Principal Component Analysis (PCA) is an unsupervised technique that shows the relationships among groups of data to be determined (Stamova et al., 2009). The initial PCA plot did not show any differentiation among our samples because the variability among the 6-biological replicates was high. These outliers were removed – two replicates from each treatment group, and the PCA re-plotted (Fig 1). 'Control' and 'HS' treatment samples not subjected to chilling grouped together but when analogous samples were subsequently chilled they separated substantially (Fig. 1). However, because the 'Chilled' sample was the most separated from the others, it can be assumed that the composition of that sample was most disparate. The first and second Principal Components (PC1 and PC2) accounted for 74.4% of the variance in the data set, but sample separation was greatest on the second PC (PC2), which only accounted for 6% variance. This indicates that the samples were very similar.

The loading scores for the PCA were examined (Fig. 2). Since the groups were not separated on PC1, outlier-metabolites along the x-axis are unrelated to the experimental treatments and were not analyzed. However, metabolites that are outliers on the y-axis contributed most to the separation of the groups seen on PC2 (Fig. 1). These included valine, arachidic acid, arabinose, shikimic acid, 2-ketoisocaproic, citric acid and threonine. All are amino or organic acids except arabinose and may be metabolites that are important indicators of harvested tomato fruit response to low temperature storage.

Discriminant Function Analysis (DFA) was then used to determine if the samples consisting of all 6 biological replicates could be differentiated from each other. DFA is an *a priori* approach because the groups, i.e. 'HS', 'Control', 'Chilled' and 'HS + Chilled', are clearly defined before applying the analysis. This algorithm provides as



**Fig. 2.** Loading score plot indicating the impact of individual metabolites on the clustering of the PCA (Fig. 1). Solid circles indicate one of the 65 compounds for which a chemical structure has been identified. Of these, the six-labeled metabolites are those that contributed the most to the separation of the different treatments (Fig. 1).

great a separation as possible, by reducing the variability within the groups (which in this experiment were high) and maximizing variability between them (Stamova et al., 2009). The DFA pattern we obtained was similar to the PCA analysis with 4 replicates indicating that the groups could be discriminated based on GC–MS profiling and that the PCA classification was not due to bias in reducing replicates (data not shown).

Overall, even though the profiles were similar, GC–MS profiling was sensitive enough to discriminate among the non-chilled samples, the chilled samples, and the samples heat-shocked prior chilling that had attenuated chilling injury solely on the basis of metabolite changes.

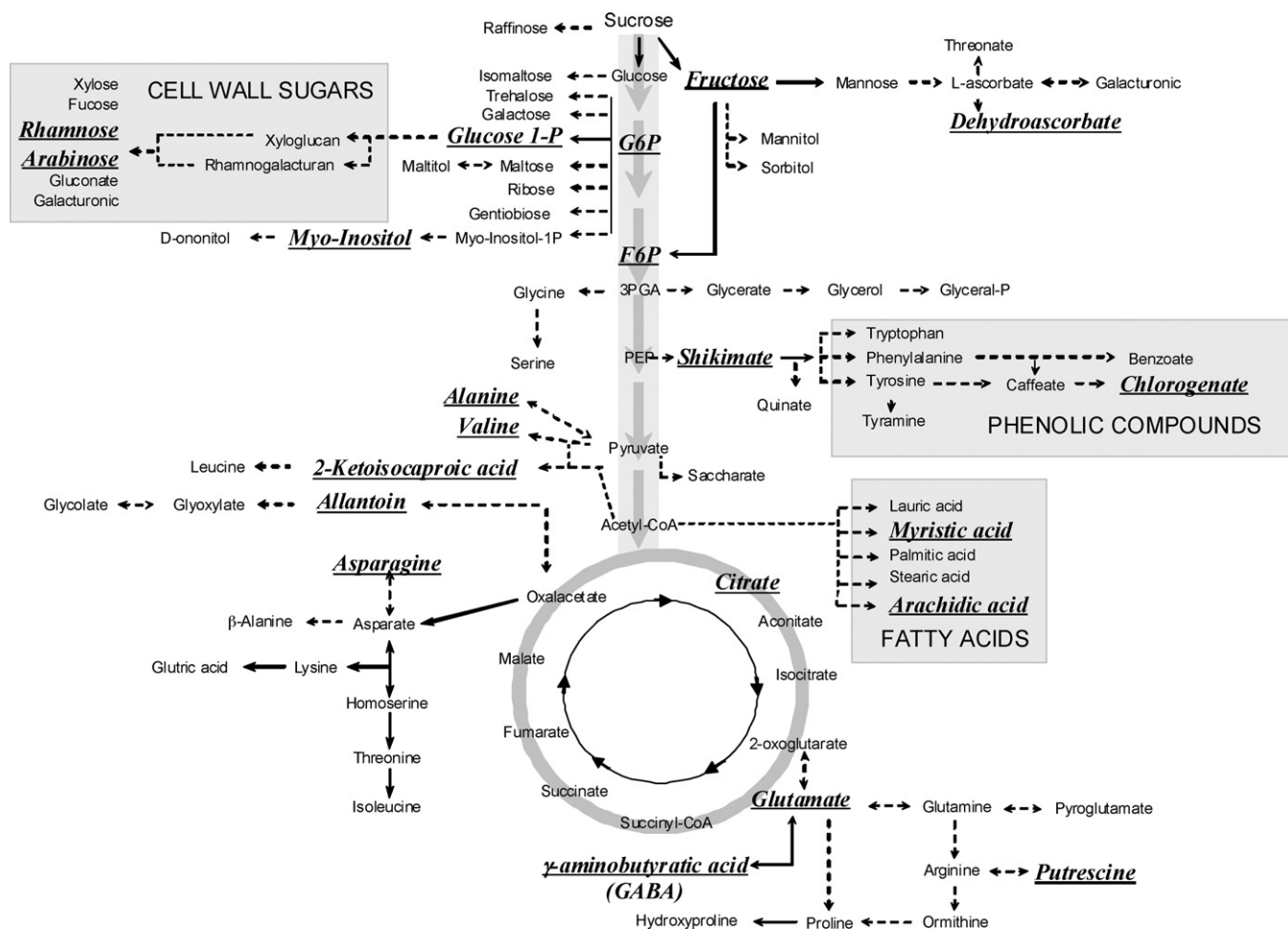
### 3.3. Metabolite changes in response to heat-shock and chilling

ANOVA and Student *t*-test, provide specific information on how each metabolite differed between treatments (Stamova et al., 2009). ANOVA has some pitfalls when used on large datasets such as those generated by metabolic profiling analysis because there can be higher chances of finding changes that may not be significant (i.e., higher false positive discovery rate; FDR) (Broadhurst and Kell, 2006). This FDR can be minimized by applying the Bonferroni Correction, which has more stringent significance confidence levels (Roessner and Beckles, 2009), however this approach was too conservative and none of our metabolites met the criteria for significance. The Student's *t*-test with the 5% significance discrimination level was used for the analysis of our data, and the False Discovery Rates are shown in Suppl. Table 1. These metabolites occurring at different levels between control and treated tissues may point to shifts in metabolism in fruit undergoing stress. They are shown in Fig. 3 and Table 1.

#### 3.3.1. Metabolites altered due to chilling

Low temperatures increased levels of arabinose, citric acid, dehydroascorbic acid, fructose-6-phosphate, glucose-6-phosphate, rhamnose, and valine (Table 1, A), while it decreased glutamic acid and shikimic acid (Table 1, B) compared to the non-treated control. Some of these changes may be discussed based on what is known about plant stress metabolism (Table 2):

- (i) Glutamic acid is a substrate for the stress-signaling molecule  $\gamma$ -aminobutyric acid (GABA) (Fig. 3), (Bouche and Fromm, 2004; Snedden and Fromm, 1999) and there is usually an inverse relationship in levels of the two (Bown and Shelp, 1997), how-



**Fig. 3.** Pathways showing the relationship among metabolites. Not all metabolites shown in the pathways were determined in our study but they are included for completeness. Treatments altered the level of the following compounds: arabinose, chlorogenate, citric acid, dehydroascorbic acid, fructose-6-P (F6P), fructose, glucose-6-P (G6P), GABA, glucose-1-P (G1P), glutamate, myo-inositol, myristic acid, putrescine, rhamnose, shikimic acid and valine.

**Table 1**  
List of the 65 metabolites identified by GC–MS from treated cv. Micro-Tom fruit.

Amino acids	Organic acids	Sugars	Fatty acids	Sugar alcohols	Others
5-Methoxytryptamine	2-Hydroxyvaleric acid	3,6-Anhydrogalactose	<u>Arachidic acid</u> C	Arabitol	<u>Allantoin</u> C,E
<u>Alanine</u> C,E	<u>2-Ketoisocaproic acid</u> C	<u>Arabinose</u> A	Lauric acid	Dodecanol	Butyrolactam
<u>Asparagine</u> E	3-Hydroxypropionic acid	<u>Fructose</u> F	<u>Myristic acid</u> E	Galactinol	N-Acetyl-D-hexosamine
Aspartic acid	Alpha ketoglutaric acid	<u>Fructose-6-phosphate</u> A,D,E	Palmitic acid	Glycerol	N-Acetyl-D-mannosamine
Beta-alanine	<u>Chlorogenic acid</u> F	Glucose	Stearic acid	<u>Myo inositol</u> C	Propane-1,3-diol
<u>GABA</u> F	<u>Citric acid</u> A,E	Glucose-1-phosphate			<u>Putrescine</u> C,E
<u>Glutamic acid</u> B	<u>Dehydroascorbate</u> A	<u>Glucose-6-phosphate</u> A,E			
Glutamine	Fumaric acid	<u>Rhamnose</u> A,C			
Glycine	Glucuronic acid	Sucrose			
Isoleucine	Lactic acid	Xylose			
Leucine	Malic acid				
Lysine	Mannonic acid				
Methionine	Pelargonic acid				
O-Acetylserine	Phosphoric acid				
Phenylalanine	Quinic acid				
Serine	Ribonic acid				
Threonine	<u>Saccharic acid</u>				
Tryptophan	<u>Shikimic acid</u> B				
Tyrosine	Threonic acid				
<u>Valine</u> A,F					

	Chilling	Heat-shock	Heat-shock + chilling
Increased	<b>A</b>	<b>C</b>	<b>E</b>
Decreased	<b>B</b>	<b>D</b>	<b>F</b>

The chilling and heat-shock treatments were compared with the control (i.e., non-chilled, non-heat-shock) treatment, while the heat-shock + chilling treatment was compared to the heat-shock treatment.

<sup>1</sup> Compounds that are underlined changed significantly ( $p < 0.05$ ) in content after being heat-shocked at 40 °C for 7 min and/or chilled at 2.5 °C for 14 d.

<sup>2</sup> Capital letters in the legend associated with the highlighted compounds indicates whether the compound increased or decreased after the specific treatment.

**Table 2**  
Known mechanistic roles of various metabolites based on published data. The metabolites listed were altered by chilling stress (14 d at 2.5 °C), heat shock treatment (40 °C) or a combination of both in this study.

Metabolites	Function	Reference
Glutamate	Substrate for stress signaling compound	Bouche and Fromm (2004), Snedden and Fromm (1999), Bown and Shelp (1997)
Dehydroascorbic acid	Defense compound against damage from various stress	Ding et al. (2001)
Arabinose	Component of the cell wall	Gross and Wallner (1979) Gomez et al. (2009)
Rhamnose	Component of the cell wall	Gross and Wallner (1979) Gomez et al. (2009)
Myo-inositol	Stress response	Loewus and Murthy (2000)
Putrescine	Stress response	Alcazar et al. (2010)
Glucose-6-phosphate	Glycolysis and oxidative pentose phosphate pathway	Dennis and Blakeley (2000)
Fructose-6-phosphate	Glycolysis and oxidative pentose phosphate pathway	Dennis and Blakeley (2000)

Harvested mature-green fruit of cv. Micro-Tom in this study.

ever in our experiment glutamate decreased with no change in GABA indicating a possible altered regulation of these steps.

- (ii) Levels of dehydroascorbic acid, the oxidation product of ascorbic acid, also increased after chilling. Ascorbic acid is a major defense compound against damage from reactive oxygen species and changes in dehydroascorbic acid may be indicative of activation of a stress response defense mechanism (Ding et al., 2001).
- (iii) The observed increases in arabinose and rhamnose – pectic polysaccharide components of the pericarp cell wall of mature-green tomatoes (Gross and Wallner, 1979) – could indicate a potential chilling-induced change in cell wall metabolism as previously documented (Gomez et al., 2009).

### 3.3.2. Metabolites altered by the HS treatment

We compared 'HS' and 'Control' fruit to identify metabolites that were altered by the heat-shock treatment alone. The heat-shock treatment induced the accumulation of alanine, allantoin, arachidic acid, 2-ketoisocaproic acid, myo-inositol, putrescine, and rhamnose (Table 1, C) while it decreased fructose-6-phosphate (Table 1, D). Myo-inositol and putrescine are induced in response to various stresses (Alcazar et al., 2010; Loewus and Murthy, 2000) and may be part of a general heat-shock-induced protective mechanism.

### 3.3.3. Metabolites altered by combinatorial temperature treatments

We compared 'HS' and 'HS+Chilling' fruit samples to identify metabolites associated with the lack of development of chilling injury symptoms. After chill-treating heat-shocked fruit, levels of alanine, allantoin, asparagine, citric acid, fructose 6-phosphate, glucose-6-phosphate, myristic acid, and putrescine increased (Table 1, E), while chlorogenate, fructose, GABA, and valine decreased (Table 1, F). Glucose-6-phosphate and fructose-6-phosphate (Fig. 3) increased in chilled fruit irrespective of whether the fruit had been heat-shocked or not, indicating that essential metabolic processes, i.e. glycolysis and the pentose phosphate pathway, are affected by chilling with or without prior shock with heat.

### 3.4. Future work: towards identifying potential metabolite biomarkers

The GC–MS data we generated can also be used to identify signature metabolites for each treatment. There are limitations to this type of analysis (i) changes in metabolite levels may not be caused by but may only coincident with a particular treatment and (ii) the robustness of these metabolites to serve as putative markers will need to be corroborated with additional experiments. We analyzed data derived as a result of the four treatments being compared simultaneously (Fig. 4) and as a result of pair-wise comparisons,

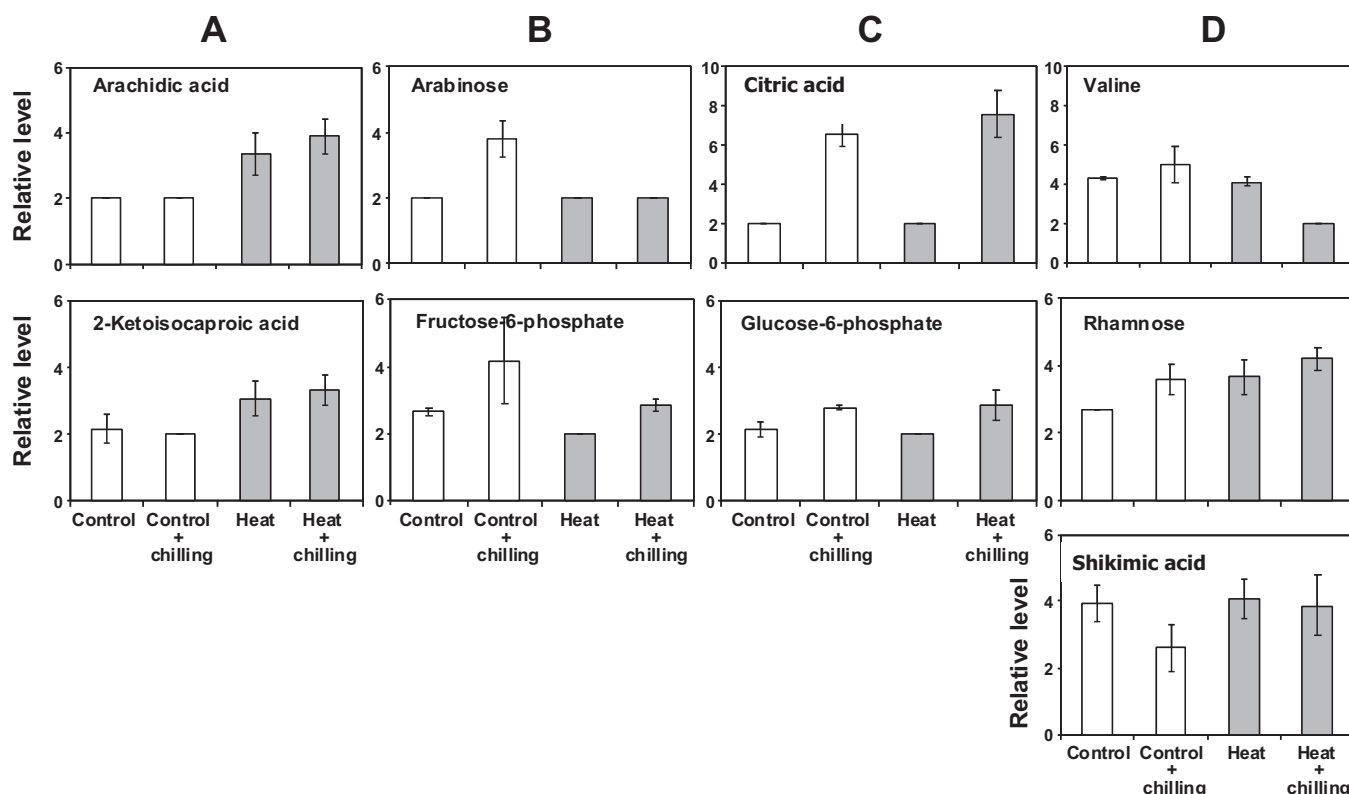
of two treatments side-by-side (Table 1). We expect overlap in the metabolites identified using both methods of analysis (Table 1), however we also expect to pinpoint specific metabolites using this approach. We organized them into four groups (A–D) as described below.

**Group 'A'** These metabolites accumulated in response to heat-shock treatment and remained at high levels in chill-tolerant fruit and may be associated with the protective function of the HS. The levels of four sugars (6% of metabolites identified), three organic acids (5%), one fatty acid (2%) and one amino acid (2%) were significantly affected by the treatments involving heat shock. The levels of two metabolites increased after 'HS' or after 'HS+Chilling', but not after chilling alone when compared to the control (Fig. 4A). One saturated fatty acid, Arachidic acid, and one organic acid, 2-ketoisocaproic acid, belonged to this group.

**Group 'B'** consisted of metabolites that increased after chilling but only if the chilled fruit had not been previously heat-shocked (Fig. 4B). The higher rate of ion leakage and respiration in control fruit after chilling compared to fruit given a prior heat-shock treatment (Luengwilai et al., 2011) suggests that changes in these metabolites may have something to do with these chilling-induced physiological changes. This group contained arabinose and fructose-6-phosphate, both of which could be investigated as physiological markers for incipient chilling injury.

**Group 'C'** consisted of metabolites that increased after chilling of both control fruit and 'HS' fruit (Fig. 4C). These metabolites may be generally associated with chilling and are not mitigated by protective high-temperature treatment. Citric acid and glucose-6-phosphate fell into this group. Chilling mature tomato fruit is known to affect their acidity; i.e. citric acid is known to increase but malic acid to decrease in mature near-ripe tomato fruit that has been chilled (Gomez et al., 2009; Kader, 1986; Thorne and Efiuvwevwe, 2006). Our results indicate that this is also true for citric acid in mature-green fruit. Interestingly, glucose-6-phosphate and fructose-6-phosphate are usually in equilibrium and metabolically linked to each other. However, we found that levels of glucose-6-phosphate and fructose-6-phosphate were altered in opposite direction depending on the treatment, indicating that fundamental perturbation of the metabolic network occur in response to low temperature stress (Steuer et al., 2003).

**Group 'D'** consisted of metabolites with levels that were significantly different in one sample as opposed to the other three but did not fit into any of the three groups previously described (Fig. 4D). After chilling, valine levels decreased in fruit that had been heat-shocked but did not change in fruit that had not been heat-shocked prior to chilling. The reduction in valine levels may therefore be associated with heat-shock-induced chilling tolerance. Shikimic acid levels decreased in chilled fruit that had not been heat-shocked but remained the same in all of the other samples. Thus, the reduction of shikimic acid occurred in fruit that were susceptible to



**Fig. 4.** Classification of metabolites measured by GC–MS from the pericarp of Micro-Tom fruit. Fruit were treated as previously described (see Section 2). The classification was performed depending on change of pattern during chilling. (A) Group A: metabolites that increased when fruit were HS and chilled, but did not change in control and chilled fruit. (B) Group B: metabolites that increased when control fruit were chilled, but did not change in HS or HS fruit after chilling. (C) Group C: metabolites that increased upon chilling (both in control and HS-fruit). (D) Group D: metabolites that show significant differences among treatments, but did not belong to any of the three groups described earlier. Values are means (after  $\log_{10}$ -transformation)  $\pm$  S.D. of 5–6 replicates.

chilling injury and it could therefore serve as a metabolite marker for incipient chilling injury. On the other hand, 'Chilling', 'HS', and 'HS+Chilling' treatments all led to changes in rhamnose levels. Since rhamnose increased irrespective of the temperature the fruit were exposed to, it would not be a useful marker for differentiating between heat and cold treatments.

Five of the seven metabolites that determined the PCA patterns (Fig. 2) valine, shikimic, citric, 2-ketoisocaproic and arachidic acid, were identified by ANOVA as differing significantly between treatments, lending support to the idea that they could be further studied as biomarkers for CI.

#### 4. Conclusion

In this paper we studied the metabolite response to chilling injury in harvested Micro-Tom tomato fruit. To our knowledge this is the first report of changes in metabolite profiles during chilling in mature green tomato fruit. GC–MS was sensitive enough to differentiate among groups of metabolites the levels of which were: (a) altered upon chilling, (b) altered upon heat-shock treatment, and (c) altered upon heat-shock- and chilling treatment (Table 1 and Fig. 4). Metabolic profiling of the same tissues that had been physiologically analyzed was critical in allowing us to put the profiling data into context. Key metabolites involved in glycolysis and the pentose phosphate pathway i.e. glucose-6-phosphate and fructose-6-phosphate were affected by chilling stress whether the fruit had been previously heat-shocked or not (Fig. 3). Heat-shock treatments appeared to have attenuated changes in the level of some metabolites, and this was associated with an alleviation of the chilling response.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.postharvbio.2011.05.014](https://doi.org/10.1016/j.postharvbio.2011.05.014).

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