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Abstract: In this study, the contents of water-soluble metabolites and mineral nutrients were measured in tomatoes cultured using organic and chemical fertilizers, with or without pesticides. Mineral nutrients and water-soluble metabolites were determined by inductively coupled plasma-atomic emission spectrometry and 1H nuclear magnetic resonance spectrometry, respectively, and results were analyzed by principal components analysis (PCA). The mineral nutrient and water-soluble metabolite profiles differed between organic and chemical fertilizer applications, which accounted for 88.0% and 55.4%, respectively, of the variation. 1H -13C-hetero-nuclear single quantum coherence experiments identified aliphatic protons that contributed to the discrimination of PCA. Pesticide application had little effect on mineral nutrient content (except Fe and P), but affected the correlation between mineral nutrients and metabolites. Differences in the content of mineral nutrients and water-soluble metabolites resulting from different fertilizer and pesticide applications probably affect tomato quality.

Dear Prof. Birch,

Please find enclosed a manuscript entitled "**Profiling Contents of Water-Soluble Metabolites and Mineral Nutrients to Evaluate the Effects of Pesticides and Organic and Chemical Fertilizers on Tomato Fruit Quality**" by Watanabe, et. al., which I am submitting for publication in *Food Chemistry*.

In this study, we demonstrated that the application of pesticides and chemical fertilizers affects the water-soluble metabolite and mineral nutrient content of tomato fruits. In particular, pesticide application had little effect on the concentrations of both inorganic elements and soluble metabolites, but did affect the correlation between the content of mineral nutrients and soluble metabolites. We used sufficient numbers of tomato fruits for the extraction of metabolites. The results of this study contribute to our understanding of the difference in quality between conventional and organically grown crops.

^{1}H NMR spectrometry is a quick and simple tool for profiling metabolites in many samples. We demonstrated the possibility of combining ^{1}H NMR profiling and mineral nutrient content for the evaluation of metabolites.

This study will contribute to the progress of research in food chemistry.

I would appreciate it if you would consider our manuscript for publication.

***Highlights (for review)**

- PCA found difference between minerals of organic tomatoes.
- PCA found difference between water-soluble metabolites of organic tomatoes.
- Pesticide application had little effect on mineral nutrients except Fe and P.
- Pesticide affected the correlation between minerals and water-soluble metabolites.
- Different fertilizer and pesticide applications probably affect tomato quality.

Profiling Contents of Water-Soluble Metabolites and Mineral Nutrients to Evaluate the Effects of Pesticides and Organic and Chemical Fertilizers on Tomato Fruit Quality

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ABSTRACT: In this study, the contents of water-soluble metabolites and mineral nutrients were measured in tomatoes cultured using organic and chemical fertilizers, with or without pesticides. Mineral nutrients and water-soluble metabolites were determined by inductively coupled plasma-atomic emission spectrometry and ^{1}H nuclear magnetic resonance spectrometry, respectively, and results were analyzed by principal components analysis (PCA). The mineral nutrient and water-soluble metabolite profiles differed between organic and chemical fertilizer applications, which accounted for 88.0% and 55.4%, respectively, of the variation. ^{1}H -^{13}C-hetero-nuclear single quantum coherence experiments identified aliphatic protons that contributed to the discrimination of PCA. Pesticide application had little effect on mineral nutrient content (except Fe and P), but affected the correlation between mineral nutrients and metabolites. Differences in the content of mineral nutrients and water-soluble metabolites resulting from different fertilizer and pesticide applications probably affect tomato quality.

KEYWORDS: *chemical fertilizers, ^{1}H -^{13}C HSQC, mineral nutrients, metabolites, NMR profiling, organic fertilizers, pesticides, tomato*

■ INTRODUCTION

Many consumers assume that organically produced fruits are healthier, with higher nutritional value, and better in quality than conventionally grown fruits. Organic production systems are believed to enhance the overall soil health and sustain agricultural and environmental quality. Therefore, organic or reduced agrochemical application systems are recommended in many developed countries. However, previous studies on food quality have produced inconsistent results (Bourn & Prescott, 2002; Zhao, Chambers, Matta, Loughin, & Carey, 2007).

Several studies have compared the effects of organic and conventional production systems on the nutritional and sensory characteristics of tomatoes. One study that focused on the nutritional content of vegetables suggested that crops produced by organic production systems may be richer in phenolics and vitamin C, while also containing fewer pesticide residues and nitrates (Rembiałkowska E, 2007; Woese. K, Lange. D, C, & W, 1997).

Other studies have demonstrated that the mean plant shoot biomass is significantly higher in plants grown with mineral nutrients, but that the concentration of total phenolics and ascorbic acid in organically grown tomatoes is much higher (Toor, Savage, & Heeb, 2006). In contrast to these findings, it has been shown that conventionally produced tomatoes have a significantly stronger flavor than organically produced tomatoes, but the overall consumer acceptance is the same for both organic and conventional tomatoes (Zhao, Chambers, Matta, Loughin, & Carey, 2007).

Consumers may not notice a sensory difference between organically and conventionally grown vegetables (Schutz & Lorenz, 1976). A similar inconsistency is apparent among trained panelists. It is difficult to study the effects of organic production on the quality of fruits because a large number of complex factors influence their quality (Clausen, Pedersen, Bertram, & Kidmose, 2011). Therefore, further studies comparing organically and conventionally grown fruit using instrumental analysis are required to reach a definitive conclusion.

The application of pesticides is an important cultivation technique, but consumers in developed countries are sometimes unwilling to eat agricultural products cultured with pesticide applications. The overuse of pesticides is dangerous for both farmers and consumers and can encourage resistance in the targeted pests. Therefore, a reduction of pesticide application is recommended worldwide. There have been no studies published regarding the effects of pesticides on metabolites in tomato fruits. In the present study, we evaluated the effects of pesticides on a large number of tomato fruits.

The term “metabolome” has been used to describe the observable chemical fingerprint of the metabolites present in whole cells, tissues, or whole organisms (Ott, Aranibar, Singh, & Stockton, 2003). Metabolomic analyses are ideally rapid, unbiased, and comprehensive. While no analytical technique meets all of these criteria, mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and infrared (IR) spectroscopy are all well established metabolomic techniques (Kruger, Troncoso-Ponce, & Ratcliffe, 2008). NMR spectroscopy is a powerful tool for analyzing and quantifying metabolite levels in cell extracts; it requires minimum preparation and handling with no derivatization (Ratcliffe & Shachar-Hill, 2001). When high-resolution NMR spectroscopy is coupled with multivariate statistical analysis, the resulting data provide useful information regarding the nutritional and genetic backgrounds of the samples (Krishnan, Kruger, & Ratcliffe, 2005; Le Gall, Colquhoun, Davis, Collins, & Verhoeyen, 2003; Mannina, Dugo, Salvo, Cicero, Ansanelli, Calcagni, et al., 2003).

Therefore, metabolic profiling provides a powerful approach for monitoring the complexity of organically and conventionally grown fruits.

We used metabolic profiling to analyze the water-soluble constituents of tomato fruits. Some metabolites that contributed to the discrimination of principal components analysis (PCA) were identified by ^{1}H -^{13}C-hetero-nuclear single quantum coherence (HSQC). The aromatic proton region (δ 5.5–9.5 ppm) was excluded from this analysis due to low signals. Our results suggest that a combination of ^{1}HNMR profiling of water-soluble metabolites with measurement of mineral nutrient contents is a useful method for evaluating the effects of different cultivation systems on metabolites from many samples.

■ MATERIALS AND METHODS

Tomato Culture. Seeds of tomato (*Solanum lycopersicum* L. cv. Momo) were sown in seed trays containing a peat and vermiculite mixture. At the third true-leaf stage, seedlings were transplanted to 10-L pots containing a mixture of Kuroboku soil, vermiculite, and leaf mold (5:1:1). The pH and EC of the soil mixture were 5.6 and 22.3

mS/mL, respectively. The pots were covered with bird nets and placed in an open space for cultivation.

The fertilizer used in the conventional production system was a slow-release fertilizer [Vegetable Eidoball (10-10-10); Sumitomo Chemical Garden Products Inc., Tokyo, Japan] and was applied basally at a rate of 23.1 g per pot. At 4 and 8 weeks after transplanting, additional fertilizer was applied at a rate of 7.7 g of Vegetable Eidoball per pot.

The organic production system was fertilized basally with a commercial rapeseed meal [$N–P_2O_5–K_2O$ (7-2-4)] at a rate of 62.6 g per pot and a commercial fish meal [$N–P_2O_5–K_2O$ (7-6-0); Asahi Industries Co., Ltd. Tokyo, Japan] at a rate of 62.6 g per pot. To adjust N, P_2O_5, and K_2O content between the conventional and organic production systems, pure potassium chloride at a rate of 5.2 g per pot was added to ensure against potassium deficiency. The efficiency index was assumed to be N 50%, P_2O_5 80%, and K_2O 90% for rapeseed meal, and N 50% and P_2O_5 80% for fish meal.

During culture, an insecticide (2000-fold dilution of 5.0% chlorfluazuron emulsion) and a pesticide (1000-fold dilution of 40.0% tetrachloro-1,3-benzenedicarbonitrile) were applied twice and once, respectively.

Four plants were used for each treatment. The tomato plants were topped after the development of the 4th truss. The fruits were harvested at the red stage as confirmed by a standard color chart (R255). They were weighed, and a Brix determination (Pocket Refractometer APAL-1; ASONE, Tokyo, Japan) was made on selected fruits. Remaining injury-free fruits were frozen in liquid nitrogen and freeze-dried. In total, 55–60 tomato fruits were used for extraction.

Nitrate, Mineral Nutrient, and C/N Ratio Analysis. Freeze-dried samples (100 mg) were extracted three times in 3 mL of 70°C distilled water. The suspensions were filtered through paper filters (No. 2; Whatman, Maidstone, Kent, UK) and fixed at 10 mL. Nitrate content was determined using a previously described method (Chuley & West, 1975).

Freeze-dried samples (400 mg) were placed in Teflon vessels, and 2.5 mL concentrated nitrate was added. The closed vessels were kept for 30 min at room temperature and incubated for 2 h at 170°C. After cooling, the digests were fixed at 50 mL with Milli Q water (Millipore, Billerica, MA, USA) and analyzed by inductively coupled plasma (ICP)-atomic emission spectrometry (ICPS-1000IV; Shimadzu, Kyoto, Japan).

Freeze-dried samples (100 mg) were placed on an autosampler (CN-CODER MT 200: Yanaco, Tokyo, Japan), and the C/N ratio was determined according to the manufacturer’s protocol.

NMR Spectroscopy. Freeze-dried powder (100 mg) was homogenized in an Auto-Mill TK AM4 (Tokken, Chiba, Japan). The supernatant was suspended with 10% (v/v) deuterium oxide (D_2O) and 1 mM sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. All NMR spectra were recorded with a DRX-500 spectrometer equipped with a ^1H inverse triple-resonance probe with triple-axis gradients, operating at 500.13 MHz for protons (Bruker, Billerica, MA, USA). The temperature of NMR samples was maintained at 25°C. For ^1H-NMR spectra, 32,768 data points with a spectral width of 10,000 Hz were collected into 128 transient and 1 dummy scans. Residual water signals were suppressed by a WATERGATE pulse sequence with a 1.2 s cycle time. Prior to Fourier transformation, the free induction decays were multiplied by an exponential window function corresponding to a 0.3 Hz line broadening factor. The acquired spectra were manually phased and baseline-corrected. The methods used for the NMR measurements of two-dimensional (2D) ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) and total correlation spectroscopy (TOCSY) have been described previously(Chikayama, Suto, Nishihara, Shinozaki, & Kikuchi, 2008; Date, Iikura, Yamazawa, Moriya, & Kikuchi, 2012; Kikuchi & Hirayama, 2007; Kikuchi, Shinozaki, & Hirayama, 2004; Sekiyama, Chikayama, & Kikuchi, 2010). NMR spectra were processed using NMRPipe software (Delaglio, Grzesiek, Vuister, Zhu, Pfeifer, & Bax, 1995) and were assigned using the Spin Assign program from the PRIMe Web site (http://prime.psc.riken.jp) (Akiyama, Chikayama, Yuasa, Shimada, Tohge, Shinozaki, et al., 2008; Chikayama, Sekiyama, Okamoto, Nakanishi, Tsuboi, Akiyama, et al., 2010).

Statistical Analysis. Each spectrum was binned for multivariate analysis using in-house software with a bin width of 0.036 ppm as described previously (Mochida, Furuta, Ebana, Shinozaki, & Kikuchi, 2009; Tian, Chikayama, Tsuboi, Kuromori, Shinozaki, Kikuchi, et al., 2007). The spectral region of 4.89–4.62 ppm was excluded to avoid variability due to water suppression and cross-saturation effects. The data were normalized to the total intensity of the spectral region to correct for possible differences in the signal-to-noise ratio and extraction between spectra. The data were analyzed by statistical multivariate techniques using R 2.10.1 software (www.R-project.org) and MS Excel.

The R function cancor was used to calculate the canonical correlation between metabolites and mineral nutrients.

■ RESULTS

Effects of Organic and Chemical Fertilizers and Pesticides on Yield, Brix, Mineral Nutrient, Nitrate, Total C, and N Content of Tomato Fruits. The application of chemical fertilizers increased the tomato yield 1.2-fold compared to organic fertilizers (Supplemental Table 1). A 30% increase in tomato yield was obtained with a combination of pesticides and organic fertilizers compared to organic fertilizers alone. The yield obtained with the combination of pesticides and chemical fertilizers was not significantly different from those obtained using either chemical fertilizers alone or organic fertilizers with pesticide application. A combination of chemical fertilizers and pesticides achieved the highest yield of tomato, while organic fertilizers alone achieved the lowest.

Statistical analysis revealed that pesticide application increased fruit yield, but brix was not influenced by either fertilizers or pesticides (Supplemental Table 1). The nitrate content and C/N ratio of tomato fruits was not significantly different between organic and chemical fertilizer applications (Supplemental Table 2), but there was a significant difference in the C ratios at the 5% level.

Organic and chemical fertilizers had different effects on the absorption of K, Mg, Ca, Fe, and Mn, but not P and B (Table 1). Compared to chemical fertilizers, organic fertilizers stimulated the absorption of Fe, Mn, and Zn 1.4-, 2.4-, and 1.8-fold, respectively. In contrast, the absorption of K, Mg, and Ca decreased to 90%, 89%, and 73%, respectively. Pesticide application had little effect on the absorption of mineral nutrients, with the exception of the Fe content of tomato fruits cultured by organic fertilizers, which increased by about 20% and the P content of tomato fruits cultured by chemical fertilizers, which decreased by 10%.

^{1}H NMR spectra. Figure 1A shows typical ^{1}H NMR spectra of aqueous extracts of tomato fruits. The signals in the aromatic region (δ 6.0–8.0) were smaller than those in the aliphatic or sugar region. Little difference was observed between the spectra of aqueous extracts of the fruits grown with organic and chemical fertilizer, with or without pesticides. Figure 1B and C show expanded spectra in the δ ~3.0 and δ 5.5–9.0 regions. Large signals were assigned using the Spin Assign program on the PRIMe (http://prime.psc.riken.jp).

Principal components analysis (PCA). PCA is a clustering method that reduces the dimensionality of multivariate data while preserving most of its variance (Eriksson, Johansson, Kettaneh-Wold, Trygg, Wikström, & Wold, 2006). The mineral nutrients in tomato fruits cultured using organic and chemical fertilizers with and without pesticides were subjected to PCA to outline the differences between the culture systems. As shown in Figure 2A, the contents of mineral nutrients were generally separated between organic and chemical fertilizer application by both principal component 1 (PC1) and PC2. This separation occurred in the first two principal components, which cumulatively accounted for 88.06% of the total variation. There was no indication of a separation between pesticide application and no-pesticide application using either organic or chemical fertilizers.

Figure 2B shows the loading scores of the mineral nutrients. The contents of K and Ca in tomato fruits were positively discriminating components of the PC1 scores and negatively discriminating components of the PC2 scores, respectively.

Figure 2C shows the PCA results for soluble metabolites between organic and chemical fertilizer applications with and without pesticide applications. The graph (Figure 2C), which plots PC1 scores versus PC2 scores, clearly discriminates between the metabolic profiles of tomatoes grown with organic versus chemical fertilizer, but no difference was found between pesticide application and no-pesticide application.

The loading plots of PC1, PC2, and PC3 (Figure 2D) show the contribution of the soluble metabolites to the scores. Aliphatic protons from organic acids, amino acids, and sugars contributed to the discrimination. Figures 2E and F show the variables with more than 0.1 negative and positive loading scores. PC1 scores of more than ±0.2 corresponded to resonances in the δ 3.998, 3.89, 3.71, 3.674, and 3.566 ppm signals. PC2 scores greater than ±0.2 ppm corresponded to δ 3.818 and 3.386 ppm. The PC3 scores for the δ 3.782, 3.764, 3.530, 3.458, 3.386, 2.774, and 2.666 ppm signals exceeded ±0.3 ppm. The sugar region (δ 3.5–5.5 ppm) contributed to positive PC1 and PC3 scores and negative PC2 scores, whereas the amino acid and organic acid regions (δ ~3.5 ppm) contributed to positive PC2 scores and negative PC1 and PC3 scores.

^{1}H -^{13}C HSQC spectra. To identify the signals contributing to PC1, 2, and 3 scores, ^{1}H -^{13}C HSQC spectra were obtained. Figures 3A–C show the HSQC spectra of the full region (δ ~9.0 ppm), the sugar region (δ 3.1–5.2 ppm), and the organic and amino acid region (δ ~3.1 ppm). The signals were assigned using a Web-based database (PRIMe, http://prime.psc.riken.jp). A summary of the identified signals is presented in Table 2 and Supplemental Table 3. The signal at δ 3.71 ppm that positively contributed to a high PC1 score could not be assigned.

Canonical Correlation Analysis. The R function cancor was used to calculate the correlation between the NMR signal intensity of soluble metabolites and the content of mineral nutrients in tomato fruits. The canonical variates of each production system were divided into three groups: organic and amino acid, sugar, and aromatic regions. The variates of each group were analyzed by Tukey's HSD test for comparing statistical difference in the canonical correlations among the production systems. The p values of the test are shown in Supplemental Table 4 A–C. The canonical variates of the organic and amino acid group were affected by pesticide application in combination with chemical fertilizers, but not by chemical fertilizers alone or organic fertilizers in combination with pesticides. The fertilizer type affected the canonical variates of sugar groups, but pesticide application had no significant effect on the variates. The canonical correlations between mineral nutrient contents and aromatic substances were significantly influenced by the fertilizer type and the application of pesticides, except in the combined application of chemical fertilizers and pesticides.

Figure 4 shows the correlation between the NMR signal intensity of soluble metabolites and the content of mineral nutrients in tomato fruits. While the application of organic fertilizers resulted in positive correlations between the contents of Mg, P, K, and Fe and the signal intensity of organic and amino acids, the addition of pesticide application lowered the correlations. Conversely, the application of organic fertilizers resulted in negative correlations with the content of Ca, and the addition of pesticide application increased the negative correlations. While the application of chemical fertilizers resulted in positive correlations between the contents of Mg, P, K, Mn, and Zn and the signal intensity of organic and amino acids, the addition of pesticide application increased the positive correlations with the contents of P, Mn, and Fe, and increased the negative correlations with the content of Ca. The application of organic fertilizers resulted in positive and negative correlations between the Mg, P, and K contents and the signal intensity of sugars, but the addition of pesticide application had almost no effect on these correlations. The correlations between Ca content and the signal intensity of sugars had adverse interactions with other minerals. The application of chemical fertilizers and pesticides increased the positive and negative correlations between P, Mn, and Fe contents and the signal intensity of sugars, but had almost no effect on the correlation between Ca and sugars. Large positive correlations between Mn and Fe contents and the signal intensity of each metabolite, except for some sugars (δ 3.4–4.0 ppm), and negative correlations between Ca and the metabolites were observed in tomatoes cultured with the application of chemical fertilizers and pesticides.

■ DISCUSSION

Plant metabolites consist of a large variety of materials and contain a high degree of structural complexity. Because MS combines well with gas chromatography (GC), GC-MS or GC-MS/MS techniques have been successfully developed for the determination of trace amounts of materials. The extensive use of GC-MS promotes the availability of compound libraries for many metabolites (Ratcliffe & Shachar-Hill, 2006). However, GC-MS has the disadvantage of requiring a derivatization to separate volatile compounds. NMR has the advantage that there is no need to separate the metabolites. Consequently, NMR can provide large-scale profiling in a simple manner. We used NMR to metabolically profile tomatoes cultured in different systems.

As shown in Figure 1, aromatic compounds were not fully extracted because we used a phosphate buffer for extraction. We previously used methanol for the extraction of tomato fruits and obtained higher signals for aromatic compounds (Supplemental Figure 1). Methanol extraction requires evaporation to suspend the phosphate buffer for NMR. This procedure takes a considerable amount of time and often loses extract by bumping during evaporation. Phosphate-buffered extraction is convenient when there are many samples to test because the extract can be directly used in NMR.

The tomatoes that we used were genetically homogeneous and we expected to find minimal difference among the treatments, so the number of samples was critical for the PCA of metabolites. Therefore, we abandoned methanol extraction. The combination of phosphate-buffered extraction and NMR provides a simplified method for large-scale metabolic profiling.

Large positive loading scores for PC1 were observed around δ 3.6 ppm (Figure 2D). These chemical shifts were derived from the protons from sugars. Therefore, PC1 represented the difference in the sugar content. Loading scores of PC2 were broadly distributed from δ 2 ppm to δ 4 ppm. The positive scores ranged from δ 2 ppm to δ 3 ppm, where protons were assigned to amino and organic acids. In cherry juice, malic acid was the most important metabolite contributing to sweetness and sourness (Clausen, Pedersen, Bertram, & Kidmose, 2011). However, malic acid in tomato fruits was not a factor discriminating PC1 and PC2 scores. Differences in the sugar, amino acid, and organic acid content may affect the quality of tomatoes cultured with different systems.

The application of pesticides increased the tomato yield by 29% and 14% with the application of organic and chemical fertilizers, respectively. Pesticides effectively contributed to the tomato yield by killing insects and microorganisms, especially when a combination of organic fertilizers was used. Tomatoes cultured by organic fertilizers were susceptible to insects and microorganisms. Therefore, pesticide application together with the application of organic fertilizers successfully raised the yield of tomato fruits.

Nitrate is reduced to nitrite, which can cause adverse effects on human and animal health. Some epidemiological studies have indicated a positive correlation between the intake of nitrate and nitrite and gastric cancer in humans (Dutt, Lim, & Chew, 1987). Nitrate absorption and its accumulation in plants are influenced by a variety of factors, particularly by the excessive application of nitrogen fertilizers. Although farmers may be encouraged to reduce the amount of nitrogen fertilizer applied to crops, they are unlikely to reduce overall nitrogen applications due to the risk of lower yields.

The application of chemical fertilizers had a minimal affect on the content of nitrate and total N in tomato fruits (Supplemental Table 2), whereas the total C content increased in tomatoes following the application of organic fertilizers. This suggests that the correct application of chemical fertilizers suppresses the absorption of excess nitrogen and maintains nitrate at the appropriate level.

The application of organic fertilizers better facilitates the conversion of carbon dioxide to organic carbon (carbon dioxide assimilation) than the application of chemical fertilizers. This is reflected by the high total C content of tomato fruits cultured with organic fertilizers. The application of organic fertilizers also stimulated the absorption of essential trace elements such as Fe, Mn, and Zn (Table 1). Organic fertilizers nourish the microorganisms in the rhizosphere. Therefore, tomato fruits cultured using organic fertilizers will be rich in these minerals. Mineral-rich tomato fruits are important for human health. Accordingly, the application of organic fertilizers is recommended from a human health perspective. The application of organic fertilizers produced positive correlations between Mg, K, Mn, and Fe contents and NMR signal intensity of organic and amino acids (Figure 4). These mineral nutrients may promote the biosynthesis of organic and amino acids, because microelements often function in catalysis as essential cofactors of many metabolic enzymes.

While tomatoes cultured using organic fertilizers had positive correlations between Mg and K contents and the signal intensity of sugars and organic and amino acids, the application of pesticides with chemical fertilizers reduced this positive correlation and resulted in a decline in the brix value of tomato fruits. Generally, Mg and K play roles in sugar and carbohydrate production, transport, and storage. Mg and K probably stimulate the biosynthesis or transport of sugar and contribute to a higher brix value in tomatoes cultured using organic fertilizers. Although slight increases in the content of Mg and K were observed with the application of chemical fertilizers and pesticides (Table 1), they did not affect brix values. The application of pesticides had an adverse effect on brix values. The correlation between mineral nutrient content and the signal intensity of water-soluble aromatic metabolites was significantly affected by the combination of organic fertilizers and chemical fertilizers with pesticides (Supplemental Table 4C). The application of chemical fertilizers and pesticides increased the correlation between the aromatic metabolites and the B, Mn, and Fe contents, which would differ in the content of water-soluble secondary metabolites. The combination of

^{1}H NMR profiling and ICP analysis with canonical correlation analysis provides a new method for elucidating the role of mineral nutrients in metabolomics.

In conclusion, pesticide application only minimally affected the content of mineral nutrients, but influenced the correlation between mineral nutrients and soluble metabolite levels in tomato fruits. The difference in the correlation is a reflection of changes in the biosynthesis of metabolites in response to pesticide application and probably makes tomato fruits taste different and influences other quality parameters.

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Figure captions

Figure 1. ^{1}H NMR spectra of soluble metabolites in tomato fruits cultured with organic fertilizer. The whole ^{1}H NMR spectra (A) and the extended spectra of δ 0.7 to 3.1 ppm (B) and δ 5.3 to 9.2 ppm (C). The signals were assigned using the Spin Assign program on the PRIMe website (http://prime.psc.riken.jp).

Figure 2. A) Sample scores for the first (PC1) and second (PC2) principal components provided by principal component analysis for mineral nutrients in tomato fruits. Each group consisted of 35 to 40 tomato samples. B) Loading scores for the first (PC1) and the second (PC2) principal components. C) Sample scores for the first (PC1) and second (PC2) principal components provided by principal component analysis for soluble metabolites in tomato fruits. Each group consisted of 50 to 60 tomato samples. D) Principal component analysis loadings of soluble metabolites with the first principal component (PC1), the second (PC2) and the third (PC3) in tomato fruits. PCA loadings of soluble metabolites with PC1 (E) and PC2 (F). The loading scores more than ± 0.1

were shown. The signals were assigned using the Spin Assign program on the PRIMe website (http://prime.psc.riken.jp).

Figure 3. The ^{1}H-^{13}C HSQC spectra (δ-0.5 to 9.5 ppm) of soluble metabolites in tomato fruits cultured with organic fertilizers. A) The whole ^{1}H-^{13}C HSQC spectra, B) the extended figure of δ 3.1 to 5.3 ppm and C) the extended figure of δ 0.9 to 3.1 ppm. The numbered signals were assigned using the Spin Assign program on the PRIMe website (http://prime.psc.riken.jp). The assigned metabolites were shown in supplemental table 3.

Figure 4. Correlation between ^{1}H NMR intensity of soluble metabolites and contents of mineral nutrients in tomato fruits.

Table(s)

Table 1 Content of mineral nutrients of tomato fruits

Application	P (mg/DW)	K (mg/DW)	Mg (mg/DW)	Ca (mg/DW)	Fe (mg/DW)	Mn (mg/DW)	B (mg/DW)	Zn (mg/DW)
Organic fertilizers	3.921a ± 0.609	17.140a ± 1.958	2.148a ± 0.350	1.561a ± 0.534	1.369a ± 0.312	0.695a ± 0.187	0.218a ± 0.114	0.243a ± 0.164
Organic fertilizers and pesticides	4.004a ± 0.575	17.761a ± 1.449	2.287a,b ± 0.255	1.465a ± 0.406	1.150b ± 0.167	0.671a ± 0.163	0.221a ± 0.087	0.200a,b ± 0.087
Chemical fertilizer	4.212a ± 0.829	19.011b ± 1.251	2.407a,b ± 0.353	2.137b ± 0.507	0.985b,c ± 0.337	0.285b ± 0.063	0.277a ± 0.168	0.133a,b ± 0.051
Chemical fertilizers and pesticides	4.728b ± 0.544	19.233b ± 1.406	2.343a ± 0.212	1.974b ± 0.555	0.910c ± 0.225	0.296b ± 0.055	0.201a ± 0.027	0.201a ± 0.121

Data are means ± SD (n = 20 ~ 40). Values followed by the same letters are not significantly different at the 5% level using Tukey's HSD test.

Table 2 Sumarry of metabolites contriibuting PC1 and PC2 scores

^{1}H ppm	4.034	3.96	3.89	3.674	3.566	4.034	3.89	3.746	3.674	3.962	3.854	3.818	3.494	3.386	3.818	3.494	3.458	3.53	3.494	3.242	2.702	2.81	2.126	2.414	2.63
Candidate materials	D-Fructose					Raffinose				D-Galactose	D-Ribose	D-Glucose			Gentiobiose			Cellobiose		L-Arginine	L-Aspartate		L-Glutamine		Citrate

The signals were identified by ^{1}H NMR and ^{1}H-^{13}C HSQC and assigned using the Spin Assign program on the PRIMe website (http://prime.psc.riken.jp).

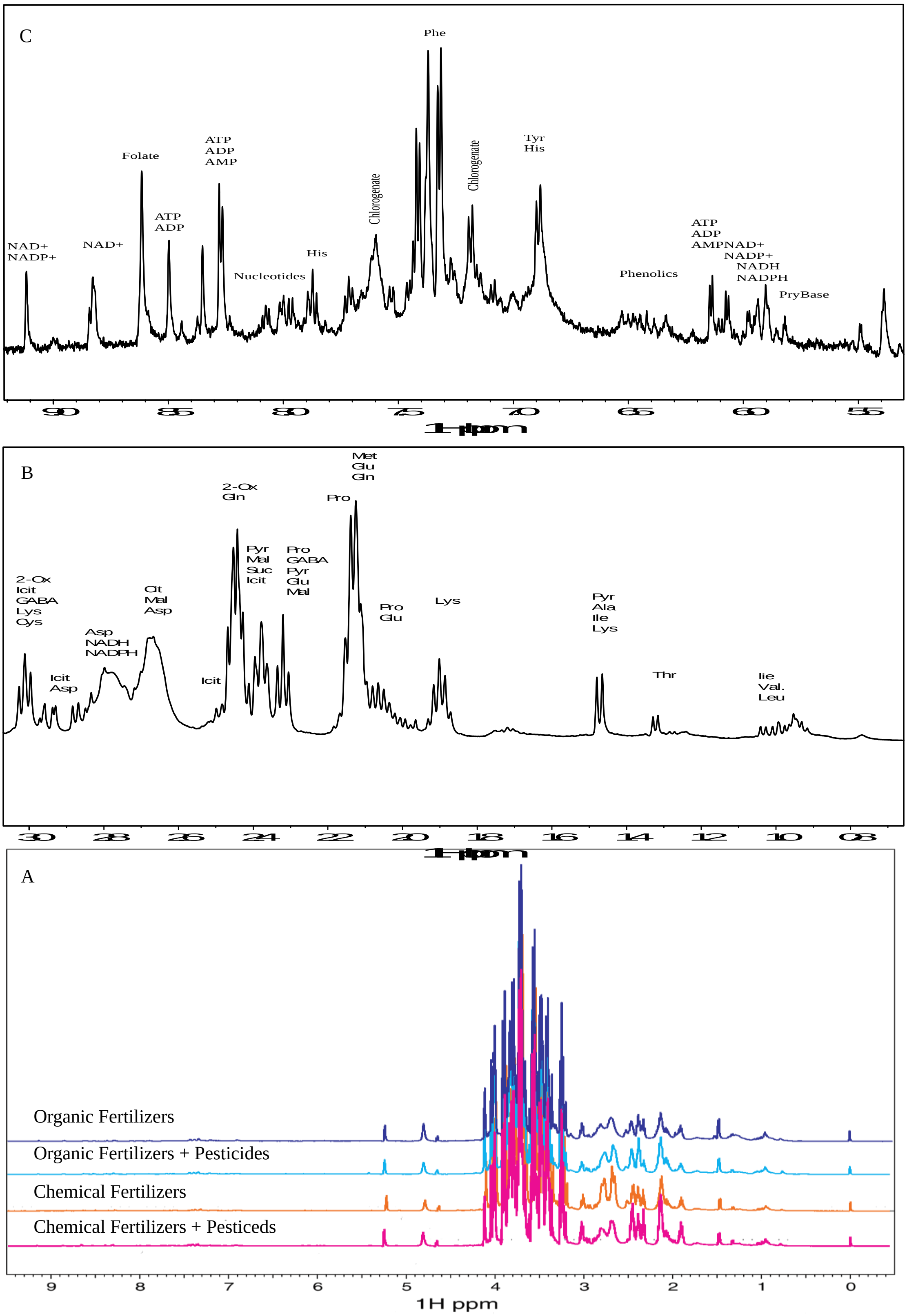

C
Phe
NAD+
NADP+
NAD+
Folate
ATP
ADP
ATP
ADP
AMP
Nucleotides
His
Chlorogenate
Chlorogenate
Tyr
His
Phenolics
ATP
ADP
AMPNAD+
NADP+
NADH
NADPH
PryBase
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
1H ppm
B
2-Ox
Icit
GABA
Lys
Cys
Icit
Asp
Asp
NADH
NADPH
Cit
Mal
Asp
Icit
2-Ox
Gln
Pyr
Mal
Suc
Icit
Pro
GABA
Pyr
Glu
Mal
Pro
Met
Glu
Gln
Pro
Glu
Lys
Pyr
Ala
Ile
Lys
Thr
Ile
Val.
Leu
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
1H ppm
A
Organic Fertilizers
Organic Fertilizers + Pesticides
Chemical Fertilizers
Chemical Fertilizers + Pesticeds
9
8
7
6
5
4
3
2
1
0
1H ppm

Fig. 1

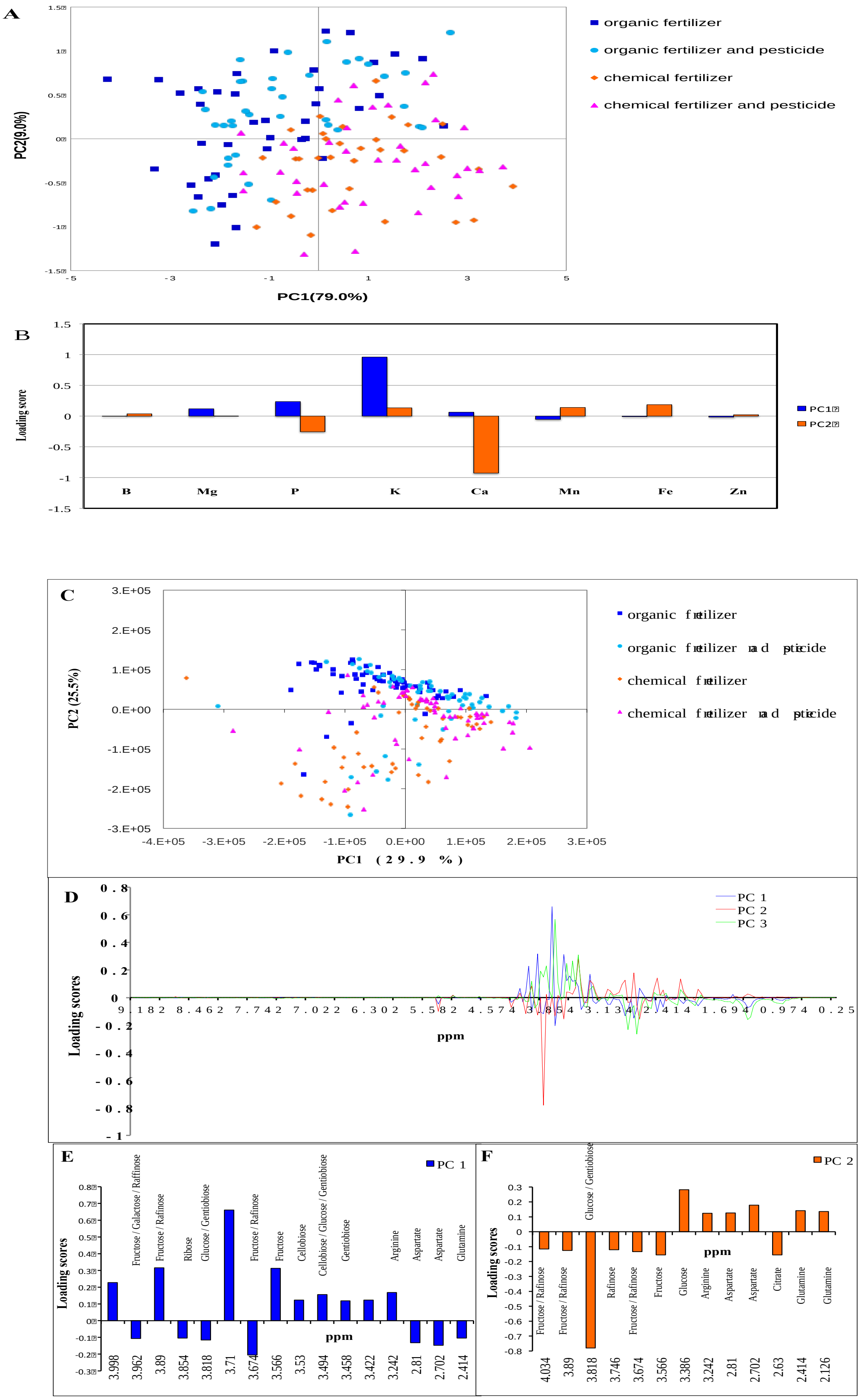
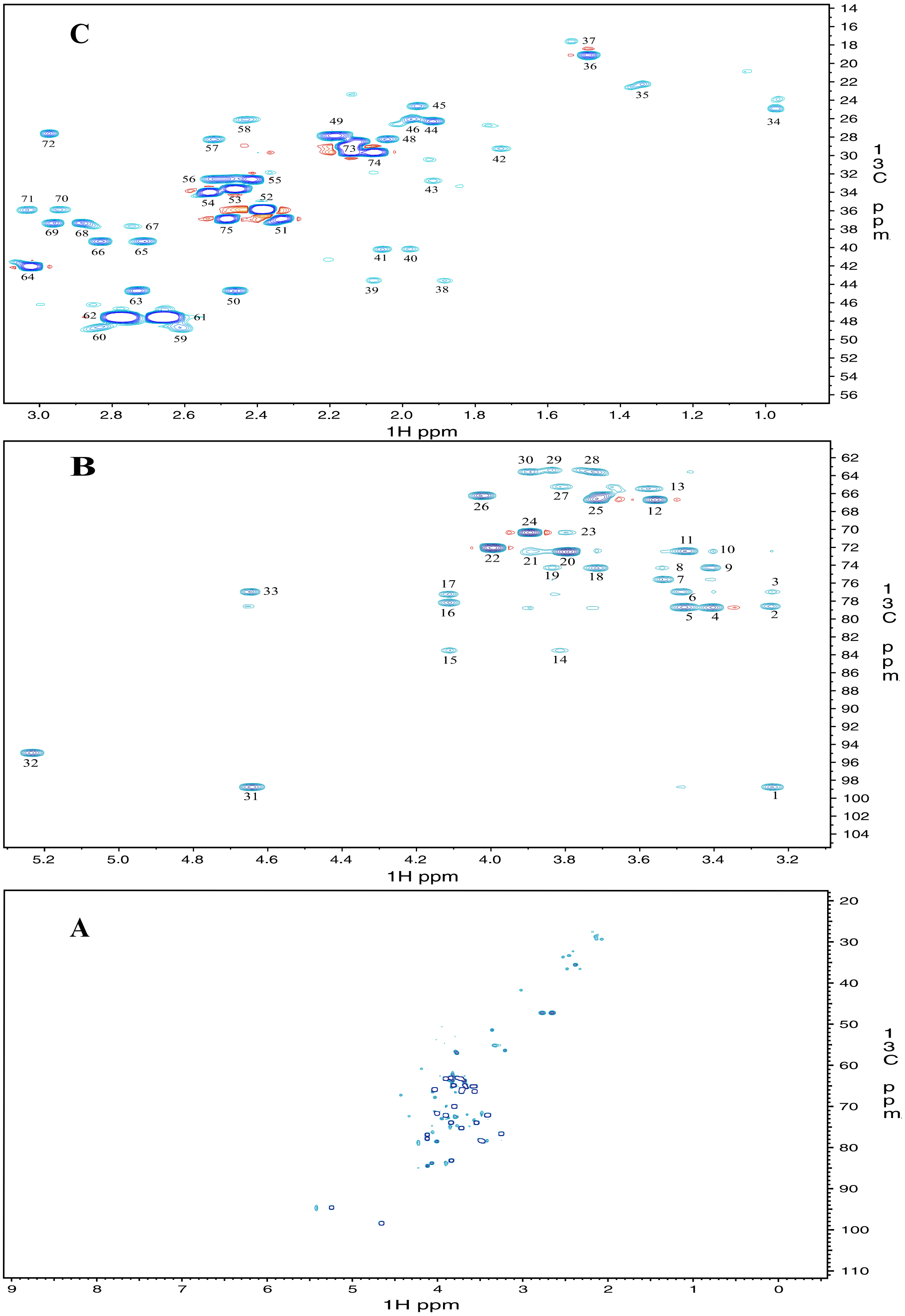

A
1.5
1
0.5
0
-0.5
-1
-1.5
-5
-3
-1
1
3
5
PC2(9.0%)
PC1(79.0%)
organic fertilizer
organic fertilizer and pesticide
chemical fertilizer
chemical fertilizer and pesticide
B
Loading score
1.5
1
0.5
0
-0.5
-1
-1.5
B
Mg
P
K
Ca
Mn
Fe
Zn
PC1
PC2
C
3.E+05
2.E+05
1.E+05
0.E+00
-1.E+05
-2.E+05
-3.E+05
-4.E+05
-3.E+05
-2.E+05
-1.E+05
0.E+00
1.E+05
2.E+05
3.E+05
PC2 (25.5%)
PC1 (29.9 %)
organic fertilizer
organic fertilizer and pesticide
chemical fertilizer
chemical fertilizer and pesticide
D
Loading scores
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
9.182
8.462
7.742
7.022
6.302
5.582
4.574
3.854
3.134
2.414
1.694
0.974
0.25
ppm
PC 1
PC 2
PC 3
E
PC 1
Loading scores
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
Fructose / Galactose / Raffinose
Fructose / Rafinose
Ribose
Glucose / Gentiobiose
Fructose / Rafinose
Fructose
Cellobiose
Cellobiose / Glucose / Gentiobiose
Gentiobiose
Arginine
Aspartate
Aspartate
Glutamine
ppm
3.998
3.962
3.89
3.854
3.818
3.71
3.674
3.566
3.53
3.494
3.458
3.422
3.242
2.81
2.702
2.414
F
PC 2
Loading scores
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
Glucose / Gentiobiose
Fructose / Rafinose
Fructose / Rafinose
Rafinose
Fructose / Rafinose
Fructose
Glucose
Arginine
Aspartate
Aspartate
Citrate
Glutamine
Glutamine
ppm
4.034
3.89
3.818
3.746
3.674
3.566
3.386
3.242
2.81
2.702
2.63
2.414
2.126

Fig. 2


C
37
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46 44
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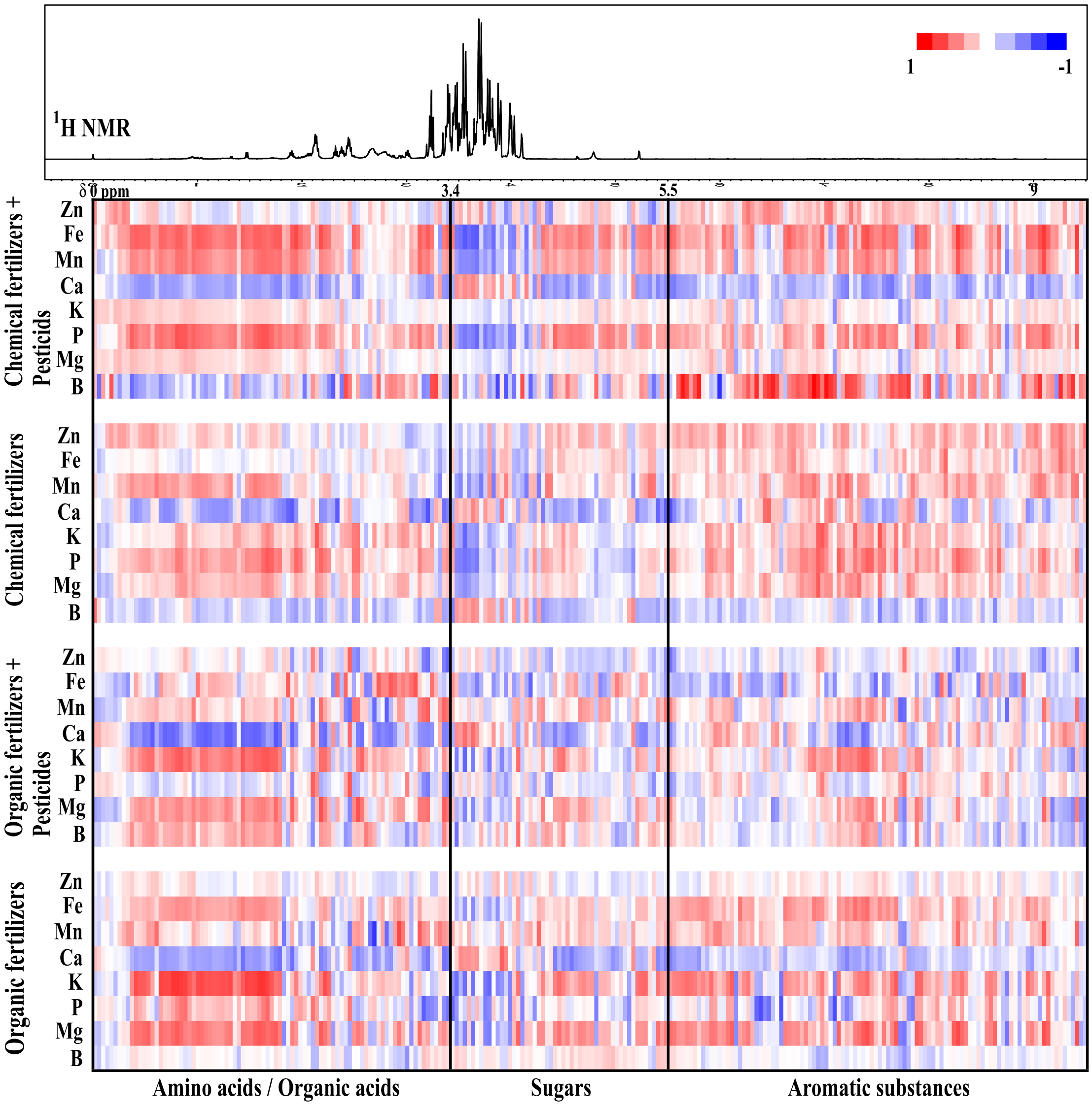
38
63
50
62
61
60
59
3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
1H ppm
14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56
13C ppm
B
30 29 28
13
27
26
25
12
24
23
11
22
21
20
10
8
9
19
18
7
17
33
3
6
16
5
4
2
15
14
32
31
1
5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2
1H ppm
62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96 98 100 102 104
13C ppm
A
9 8 7 6 5 4 3 2 1 0
1H ppm
20 30 40 50 60 70 80 90 100 110
13C ppm

Fig. 3



^{1}H NMR
1
-1
δ 0 ppm
3.4
5.5
9
Chemical fertilizers + Pesticides
Chemical fertilizers
Organic fertilizers + Pesticides
Organic fertilizers
Zn
Fe
Mn
Ca
K
P
Mg
B
Amino acids / Organic acids
Sugars
Aromatic substances

Fig. 4

Supplemental Table 1 Effects of fertilizers and pesticides on yield and brix of tomato fruits

Application	Yield (g) / plant	Brix(%) / fruit
Organic fertilizers	421.3a ± 21.5	9.3a ± 1.3
Organic fertilizer and pesticides	545.4b ± 11.1	9.2a ± 0.9
Chemical fertilizer	494.2a,b ± 48.7	9.1a,b ± 1.1
Chemical fertilizers and pesticides	565.1b ± 46.3	8.7b ± 1.1

Data are means ± SD (n = 20 - 40). Values followed by the same letters are not significantly different at the 5% level using Tukey's HSD test.

Supplemental Table 2 Nitrate, total C and N content of tomato fruits

Fertilizer Application	Nitrate (mmol/g DW)	C (%)	N (%)
Organic	0.122 ± 0.045	40.233 ± 0.266	1.903 ± 0.113
Inorganic	0.148 ± 0.046	39.855 ± 0.377	1.890 ± 0.078
p-value (t.test)	0.22	0.036	0.801

Supplemental Table 3 ^{1}H-^{13}C HSQC NMR signal assignment of tomatoes cultured by organic fertilizers

Peak No.	QRY_PPM1H	QRY_PPM13C	DB_PPM1H	DB_PPM13C	DB_INTENSITY	DB UNIQ	METABOLITE	KEGGID	DETECTION	dPPM1H	dPPM13C	P_VALUE
1	7.32	132.1	7.318	131.968	0	0.5	L-Phenylalanine	C00079	0.333333333	0.002	0.132	2.30E−07
2	7.42	132.1	7.414	131.919	0	0.166666667	L-Phenylalanine	C00079	0.333333333	0.006	0.181	3.60E−15
3	7.42	130.7	7.386	130.7460337	7002639	0.5	(R)-Mandelate	C01983	0.25	0.034	−0.046	1.30E−81
4	7.38	120.4	unknown									
5	6.89	118.8	6.888	118.496	0	0.25	L-Tyrosine	C00082	0.2	0.002	0.304	1.00E−34
6	7.12	119.3	7.13	119.557	0	0.5	L-Histidine	C00135	0.125	−0.01	−0.257	9.50E−32
7	7.18	118.8	unknown									
8	7.2	117.9	7.177	117.6050337	22400000	1	Chlorogenate	C00852	0.076923077	0.023	0.395	2.70E−94
9	5.82	110.5	unknown									
10	5.9	105.2	5.885	104.912	1070000	0.5	Uridine	C00299	0.125	0.015	0.288	1.40E−46
10	5.9	105.2	5.878	104.7440337	37300000	0.5	Deoxyuridine	C00526	0.125	0.022	0.456	4.20E−110
11	7.89	105.2	unknown									
12	5.49	99.8	unknown									
13	5.47	97.6	unknown									
14	5.39	95	5.397	94.813	0	0.333333333	Sucrose	C00089	0.090909091	−0.007	0.187	7.10E−17
15	5.61	95	unknown									
16	5.75	95	unknown									
17	5.82	95	unknown									

18	5.53	91.9	unknown									
19	5.9	92.2	5.897	91.972	1810000	1	Uridine	C00299	0.125	0.003	0.228	2.60E−20
20	5.98	91.3	5.963	91.432	1700000	0.333333333	UDP	C00015	0.142857143	0.017	−0.132	4.10E−27
20	5.98	91.3	5.991	91.029	1400000	0.333333333	UMP	C00105	0.125	−0.011	0.271	6.70E−36
20	5.98	91.3	5.998	91.819	1460000	0.333333333	CTP	C00063	0.142857143	−0.018	−0.519	2.00E−121
21	6.06	91.2	unknown									
22	6.12	89.9	6.131	89.993	1730000	0.25	IMP	C00130	0.142857143	−0.011	−0.093	2.90E−12
22	6.12	89.9	6.116	89.667	0	0.142857143	NADH	C00004	0.058823529	0.004	0.233	1.20E−21
22	6.12	89.9	6.131	89.669	0	0.076923077	ADP	C00008	0.166666667	−0.011	0.231	1.40E−28
22	6.12	89.9	6.125	89.381	10334840	0.111111111	AMP	C00020	0.142857143	−0.005	0.519	1.10E−100
22	6.12	89.9	6.134	89.363	0	0.071428571	ATP	C00002	0.142857143	−0.014	0.537	1.70E−119
23	3.24	98.8	unknown									
24	3.48	98.9	unknown									
25	4.65	98.4	4.639	98.62	0	0.142857143	D−Glucose	C00031	0.076923077	0.011	−0.22	9.10E−27
25	4.65	98.4	4.632	98.601	1163787	0.166666667	D−Glucuronate	C00191	0.1	0.018	−0.201	6.40E−38
26	3.52	100	unknown									
27	5.23	95	5.234	94.882	909261.8	0.111111111	D−Glucuronate	C00191	0.1	−0.004	0.118	6.50E−07
27	5.23	95	5.225	94.803	0	0.111111111	D−Glucose	C00031	0.076923077	0.005	0.197	1.30E−16
27	5.23	95	5.18	94.992	1613117	0.333333333	D−Xylose	C00181	0.1	0.05	0.008	5.00E−174
28	3.66	83.6	unknown									

29	3.79	83.6	3.818	83.339	2970000	1	D−Fructose	C00095	0.0625	−0.028	0.261	6.50E−80
30	4	84.1	4.038	83.961	1310000	1	D−Fructose	C00095	0.125	−0.038	0.139	7.40E−108
31	4.11	84.9	4.103	84.745	1580000	0.25	D−Fructose	C00095	0.0625	0.007	0.155	6.90E−13
31	4.11	84.9	4.082	85.005	0	0.333333333	NADH	C00004	0.058823529	0.028	−0.105	4.30E−59
32	4.11	83.6	unknown									
33	3.24	78.6	unknown									
34	3.4	78.7	3.422	78.572	2038156	0.5	D−Xylose	C00181	0.1	−0.022	0.128	3.00E−40
35	3.48	78.7	3.474	78.487	0	0.125	D−Glucose	C00031	0.153846154	0.006	0.213	9.10E−20
35	3.48	78.7	3.452	78.652	0	0.2	D−Glucose	C00031	0.153846154	0.028	0.048	6.60E−56
35	3.48	78.7	3.498	78.377	1019776	0.166666667	D−Glucuronate	C00191	0.1	−0.018	0.323	3.10E−61
36	3.72	78.7	3.716	78.925	1146209	1	D−Glucuronate	C00191	0.1	0.004	−0.225	2.70E−20
37	3.9	78.9	unknown									
38	4	78.9	unknown									
39	4.05	78.7	unknown									
40	4.11	78	4.104	78.143	5330000	0.5	D−Fructose	C00095	0.0625	0.006	−0.143	1.10E−10
41	4.66	78.6	unknown									
42	3.24	77	3.235	76.826	0	0.166666667	D−Glucose	C00031	0.076923077	0.005	0.174	1.70E−13
42	3.24	77	3.217	76.843	2224533	0.25	D−Xylose	C00181	0.1	0.023	0.157	2.20E−46
42	3.24	77	3.264	76.851	6535513	0.111111111	Maltose	C00208	0.047619048	−0.024	0.149	9.50E−49
43	3.39	77	unknown									

44	3.48	77	unknown									
45	3.82	78.3	unknown									
46	3.82	77.3	unknown									
47	4.11	77.3	4.105	77.173	4910000	0.5	D-Fructose	C00095	0.0625	0.005	0.127	2.40E-08
48	4.64	77	unknown									
49	3.41	75.7	unknown									
50	3.54	75.5	unknown									
51	3.7	75.6	3.706	75.498	0	0.1	D-Glucose	C00031	0.076923077	-0.006	0.102	5.10E-07
51	3.7	75.6	3.701	75.418	8008382	0.090909091	Maltose	C00208	0.142857143	-0.001	0.182	7.10E-13
51	3.7	75.6	3.713	75.358	604757.1	0.090909091	D-Glucuronate	C00191	0.1	-0.013	0.242	8.40E-34
51	3.7	75.6	3.675	75.676	6538242	0.090909091	Maltose	C00208	0.142857143	0.025	-0.076	3.70E-46
51	3.7	75.6	3.67	75.62	2303649	0.111111111	Maltose	C00208	0.142857143	0.03	-0.02	3.00E-63
52	3.84	75.7	3.84	75.774	1110000	0.5	D-Sorbitol	C00794	0.142857143	0	-0.074	0.01
52	3.84	75.7	3.865	75.538	47600000	0.333333333	Chlorogenate	C00852	0.076923077	-0.025	0.162	1.30E-53
53	3.4	74.2	unknown									
54	3.54	74.2	3.548	74.139	2131060	0.04	Maltose	C00208	0.095238095	-0.008	0.061	0.0000016
54	3.54	74.2	3.554	74.197	2162284	0.04	Maltose	C00208	0.095238095	-0.014	0.003	2.60E-14
54	3.54	74.2	3.526	74.162	0	0.047619048	D-Glucose	C00031	0.076923077	0.014	0.038	7.70E-15
54	3.54	74.2	3.514	74.208	1308587	0.045454545	D-Xylose	C00181	0.1	0.026	-0.008	1.30E-47
54	3.54	74.2	3.565	74.1	765632.9	0.071428571	D-Glucuronate	C00191	0.1	-0.025	0.1	1.10E-47

54	3.54	74.2	3.544	73.782	0	0.076923077	Sucrose	C00089	0.090909091	−0.004	0.418	1.50E−65
55	3.7	74.3	3.72	73.836	112000000	0.2	L−Iditol	C01507	0.25	−0.02	0.464	5.60E−107
56	3.7	74.3	3.72	73.836	112000000	0.2	L−Iditol	C01507	0.25	−0.02	−0.094	1.80E−54
56	3.81	74.3	3.823	74.163	0	0.071428571	D−Glucose	C00031	0.076923077	−0.013	0.137	2.80E−19
57	5.23	74.3	unknown									
58	3.23	72.4	unknown									
59	3.38	72.4	3.399	72.292	0	0.166666667	D−Glucose	C00031	0.076923077	−0.019	0.108	5.30E−30
60	3.46	72.4	unknown									
61	3.7	72.3	unknown									
62	3.78	72.4	3.777	71.898	25200000	1	Mannitol	C00392	0.25	0.003	0.502	2.90E−93
63	3.87	72.43	3.885	72.439	8920000	0.166666667	D−Fructose	C00095	0.0625	−0.015	−0.009	2.40E−16
63	3.87	72.43	3.841	72.44	1320000	0.25	D−Sorbitol	C00794	0.142857143	0.029	−0.01	4.70E−59
64	3.99	72	3.99	71.938	17100000	0.2	D−Fructose	C00095	0.0625	0	0.062	0.04
65	3.78	70.4	3.792	70.306	7030000	1	D−Fructose	C00095	0.0625	−0.012	0.094	6.30E−14
66	3.87	70.4	unknown									
67	4	70.4	unknown									
68	3.56	66.7	3.555	66.631	6340000	0.5	D−Fructose	C00095	0.0625	0.005	0.069	0.00034
69	3.7	66.6	3.705	66.625	7510000	0.5	D−Fructose	C00095	0.0625	−0.005	−0.025	0.011
70	4.01	66.1	4.012	66.022	11000000	0.142857143	D−Fructose	C00095	0.0625	−0.002	0.078	0.0032
70	4.01	66.1	4.006	66.201	22094120	0.166666667	AMP	C00020	0.142857143	0.004	−0.101	0.000015

70	4.01	66.1	3.993	66.054	1490000	0.166666667	UMP	C00105	0.125	0.017	0.046	1.60E−21
70	4.01	66.1	4.012	66.358	3420000	0.2	GMP	C00144	0.2	−0.002	−0.258	2.80E−25
70	4.01	66.1	3.991	65.708	1590000	0.166666667	CMP	C00055	0.142857143	0.019	0.392	8.60E−82
71	3.58	65.4	3.567	65.467	2810000	0.25	D−Fructose	C00095	0.0625	0.013	−0.067	4.50E−14
72	3.66	65.1	3.667	65.088	1460000	0.058823529	D−Fructose	C00095	0.0625	−0.007	0.012	0.00036
72	3.66	65.1	3.638	65.428	103000000	0.066666667	L−Iditol	C01507	0.5	0.022	−0.328	1.60E−73
72	3.66	65.1	3.676	65.522	106000000	0.076923077	Threonate	C01620	0.25	−0.016	−0.422	2.00E−83
72	3.66	65.1	3.63	65.359	1170000	0.066666667	D−Sorbitol	C00794	0.142857143	0.03	−0.259	1.40E−87
72	3.66	65.1	3.705	65.437	98800000	0.25	L−Iditol	C01507	0.5	−0.045	−0.337	1.70E−182
73	3.8	65.1	3.792	65.099	3590000	0.111111111	D−Fructose	C00095	0.0625	0.008	0.001	0.000037
73	3.8	65.1	3.808	65.024	0	0.090909091	Sucrose	C00089	0.090909091	−0.008	0.076	2.90E−07
73	3.8	65.1	3.826	65.618	1130000	0.166666667	D−Sorbitol	C00794	0.142857143	−0.026	−0.518	1.90E−145
74	3.39	63.5	unknown									
75	3.45	63.7	unknown									
76	3.7	63.5	3.715	63.447	0	0.1	D−Glucose	C00031	0.076923077	−0.015	0.053	2.40E−17
76	3.7	63.5	3.677	63.721	3943848	0.25	D−Xylose	C00181	0.1	0.023	−0.221	3.30E−55
76	3.7	63.5	3.666	63.977	0	0.25	Sucrose	C00089	0.090909091	0.034	−0.477	7.70E−164
76	3.7	63.5	3.747	63.754	1390000	0.066666667	D−Mannose	C00159	0.083333333	−0.047	−0.254	2.30E−177
77	3.82	63.2	3.828	63.287	0	0.076923077	D−Glucose	C00031	0.076923077	−0.008	−0.087	6.40E−08
77	3.82	63.2	3.837	63.273	11018760	0.083333333	Maltose	C00208	0.047619048	−0.017	−0.073	1.10E−22
77	3.82	63.2	3.802	62.786	0	0.111111111	Sucrose	C00089	0.090909091	0.018	0.414	1.10E−85

78	3.81	63.2	3.828	63.287	0	0.076923077	D-Glucose	C00031	0.076923077	-0.018	-0.087	6.10E-26
78	3.81	63.2	3.837	63.273	11018760	0.083333333	Maltose	C00208	0.047619048	-0.027	-0.073	3.40E-53
78	3.81	63.2	3.802	62.786	0	0.111111111	Sucrose	C00089	0.090909091	0.008	0.414	1.10E-67
78	3.89	63.5	3.892	63.473	2200000	0.083333333	Uridine	C00299	0.125	-0.002	0.027	0.29
78	3.89	63.5	3.887	63.446	0	0.1	D-Glucose	C00031	0.076923077	0.003	0.054	0.021
78	3.89	63.5	3.898	63.513	7153288	0.1	Maltose	C00208	0.047619048	-0.008	-0.013	0.000032
78	3.89	63.5	3.868	63.76	1660000	0.111111111	D-Mannose	C00159	0.083333333	0.022	-0.26	6.30E-59
79	2.7	55	unknown									
80	2.81	55	unknown									
81	2.87	54.1	unknown									
82	2.95	54.1	unknown									
83	1.48	53.4	unknown									
84	1.48	19.2	1.465	18.896	0	1	L-Alanine	C00041	0.5	0.015	0.304	5.00E-50
85	2.34	51.2	unknown									
86	2.66	48.7	2.64	48.5	0	1	Citrate	C00158	0.5	0.02	0.2	4.90E-43
87	2.83	48.9	unknown									
88	2.01	49	unknown									
89	2.64	47.6	unknown									
90	2.76	47.6	unknown									
91	2.83	46.2	unknown									

92	2.46	44.8	unknown									
93	2.7	44.8	2.677	45.314	0	0.5	(S)−Malate	C00149	0.333333333	0.023	−0.514	9.40E−134
94	1.87	43.6	1.859	43.34996627	22900000	1	Quinate	C00296	0.142857143	0.011	0.25	6.50E−32
95	2.07	43.6	2.051	43.34496627	26800000	1	Quinate	C00296	0.142857143	0.019	0.255	1.80E−49
96	1.71	42	1.703	42.537	0	1	L−Leucine	C00123	0.2	0.007	−0.537	2.70E−109
97	1.89	42.1	unknown									
98	2.32	42.1	2.3	41.955	1550000	1	Deoxycytidine	C00881	0.111111111	0.02	0.145	4.00E−36
99	3.01	42.1	2.999	41.998	0	0.2	4−Aminobutanoate	C00334	0.333333333	0.011	0.102	6.60E−13
99	3.01	42.1	3.015	41.711	0	0.111111111	L−Lysine	C00047	0.166666667	−0.005	0.389	1.20E−57
100	1.97	40.2	unknown									
101	2.05	40.2	2.033	40.03096627	32900000	0.5	Quinate	C00296	0.142857143	0.017	0.169	3.50E−31
101	2.05	40.2	2.023	39.94196627	34900000	0.5	Chlorogenate	C00852	0.076923077	0.027	−39.742	1.50E−75
102	2.7	39.4	2.672	39.268	0	1	L−Aspartate	C00049	0.333333333	0.028	0.132	2.00E−61
103	2.81	39.4	2.787	39.279	0	0.5	L−Aspartate	C00049	0.333333333	0.023	0.121	9.90E−43
104	1.89	36.9	unknown									
105	2.11	36.9	unknown									
106	2.32	36.6	2.324	36.991	2830000	1	Folate	C00504	0.125	−0.004	−0.391	1.30E−57
106	2.32	36.6	2.337	36.21	0	1	L−Glutamate	C00025	0.25	−0.017	0.39	3.10E−76
107	2.48	36.9	unknown									
108	2.87	37.5	2.86	37.21	0	1	L−Asparagine	C00152	0.333333333	0.01	0.29	2.50E−38

109	2.95	37.5	2.94	37.257	0	1	L−Asparagine	C00152	0.333333333	0.01	0.243	3.40E−29
110	3.01	36.9	unknown									
111	2.09	36	unknown									
112	2.13	36	unknown									
113	2.38	36	2.337	36.21	0	1	L−Glutamate	C00025	0.25	0.043	−0.21	5.70E−145
114	2.93	36	unknown									
115	3.01	36	unknown									
116	2.15	33.7	unknown									
117	2.44	33.7	2.444	33.579	0	0.5	L−Glutamine	C00064	0.333333333	−0.004	0.121	3.60E−07
117	2.44	33.7	2.434	33.208	0	0.5	2−Oxoglutarate	C00026	0.5	0.006	0.492	1.60E−91
118	2.52	34.1	2.526	34.01296627	67100000	0.5	Glutathione disulfide	C00127	0.142857143	−0.006	0.087	0.0000055
118	2.52	34.1	2.538	34.01696627	47000000	0.5	Glutathione	C00051	0.166666667	−0.018	0.083	1.10E−25
119	1.95	32.5	unknown									
120	2.03	32.5	unknown									
121	2.42	32.8	unknown									
122	2.52	32.6	unknown									
123	2.07	29.7	2.048	29.662	0	0.25	L−Glutamate	C00025	0.25	0.022	0.038	8.40E−35
124	2.13	29.7	2.108	29.692	0	1	L−Glutamate	C00025	0.25	0.022	0.008	2.70E−34
125	2.38	29.7	unknown									
126	2.15	29.3	2.13	28.952	0	0.333333333	L−Glutamine	C00064	0.333333333	0.02	0.348	1.30E−72

126	2.15	29.3	2.154	28.85396627	110000000	0.333333333	Glutathione disulfide	C00127	0.142857143	−0.004	0.446	2.10E−74
126	2.15	29.3	2.152	28.83196627	62400000	0.333333333	Glutathione	C00051	0.166666667	−0.002	0.468	6.70E−81
127	2.46	28.8	unknown									
128	2.01	28.2	unknown									
129	2.4	28.2	unknown									
131	2.52	27.8	unknown									
132	2.17	27.8	unknown									
132	2.97	27.7	unknown									
133	1.9	26.3	1.891	26.356	0	0.5	4−Aminobutanoate	C00334	0.333333333	0.009	−0.056	1.70E−07
134	2.32	26.3	unknown									
135	2.48	26.2	unknown									
136	3.01	26.3	unknown									
137	1.95	26.2	1.992	26.461	0	1	L−Proline	C00148	0.166666667	−0.042	−0.261	7.70E−148
138	0.97	24.9	0.953	24.791	0	1	L−Leucine	C00123	0.2	0.017	0.109	4.30E−25
139	1.95	24.6	unknown									
140	2.34	24.6	unknown									
141	0.95	23.8	0.942	23.598	0	0.5	L−Leucine	C00123	0.2	0.008	0.202	4.80E−20
142	1.34	22.2	1.314	22.174	0	1	L−Threonine	C00188	0.333333333	0.026	0.026	7.90E−48
143	1.48	19.2	1.465	18.896	0	1	L−Alanine	C00041	0.5	0.015	0.304	5.00E−50

The signals were assigned using the Spin Assign program on the PRIMe website (http://prime.psc.riken.jp) and Human Metabolome Database (http://www.hmdb.ca). QUERY PEAK=Query peak No., QUERY 1H PPM=1H chemical shift of query, QUERY 13C PPM=13C chemical shift of query, DATABASE 1H PPM=1H chemical shift of peak, DATABASE 13C PPM=13C chemical shift of peak, DATABASE INTENSITY=Intensity of peak, DATABASE UNIQ=Uniqueness, ANNOTATION METABOLITE=Metabolite annotated from peak, ANNOTATION Δ1H=Difference of query 1H ppm from DB, ANNOTATION Δ13C=Difference of query 13C ppm from DB, ANNOTATION p-value=p-value for the annotation

Supplemental Table 4 ^{1}H-^{13}C HSQC NMR signal assignment of organic tomatoes

The absolute differences of ^{1}H and ^{13}C chemical shifts show the differences between HMDB and HSQC data.

Detection "0" means the absolute differences are greater than 0.05 of 1H and 0.5 of 13C, while "1" means the other case.

Metabolites	HMDB (http://www.hmdb.ca)			HSQC Data				Detection
	Carbon NO.	1H PPM	13C PPM	1H PPM	Absolute Difference	13C PPM	Absolute Difference	
Deoxycytidine	1	7.8205	144.584	ND		ND		0
Deoxycytidine	2	6.2544	88.8954	ND		ND		0
Deoxycytidine	3	6.0349	99.2081	6.079	0.044	98.924	0.28	1
Deoxycytidine	4	4.4286	73.3247	4.427	0.002	73.46	0.14	1
Deoxycytidine	5	4.043	89.3582	4.014	0.029	89.265	0.09	1
Deoxycytidine	6	3.8354	64.0935	3.857	0.022	64.093	0.00	1
Deoxycytidine	7	3.754	64.0508					0
Deoxycytidine	8	2.4266	41.8356	2.402	0.025	42.434	0.60	0
Deoxycytidine	9	2.2938	41.8755	2.304	0.010	42.141	0.27	1
Cytidine	1	7.827	144.4946	7.868	0.041	144.73	0.24	1
Cytidine	2	6.0428	99.0669	6.079	0.036	98.924	0.14	1
Cytidine	3	5.8912	93.0211	5.902	0.011	93.216	0.19	1
Cytidine	4	4.2986	76.7476	ND		ND		0
Cytidine	5	4.196	72.0022	4.152	0.044	71.264	0.74	0
Cytidine	6	4.1173	86.5578	ND		ND		0
Cytidine	7	3.9235	63.6065	3.896	0.028	63.508	0.10	1
Cytidine	8	3.8106	63.5609	3.837	0.026	63.362	0.20	1
Cytidine monophosphate	1	8.0771	144.4489	8.006	0.071	144.877	0.43	1
Cytidine monophosphate	2	6.1259	99.2974	6.079	0.047	99.07	0.23	1
Cytidine monophosphate	3	5.9911	91.7815	5.981	0.010	91.314	0.47	1
Cytidine monophosphate	4	4.3346	77.1407	4.309	0.026	77.265	0.12	1
Cytidine monophosphate	5	4.3324	72.3881	4.309	0.023	72.581	0.19	1
Cytidine monophosphate	6	4.2332	86.0567	ND		ND		0
Cytidine monophosphate	7	4.0543	65.6324	3.955	0.099	65.411	0.22	1
Cytidine monophosphate	8	3.9769	65.5932	3.955	0.022	65.411	0.18	1
Uridine	1	7.8641	144.5578	7.868	0.004	144.73	0.17	1

Uridine	2	5.9032	92.0857	5.922	0.019	92.192	0.11	1
Uridine	3	5.8916	105.0767	5.902	0.010	105.217	0.14	1
Uridine	4	4.3468	76.4773	4.349	0.002	76.533	0.06	1
Uridine	5	4.229	72.077	4.211	0.018	72.435	0.36	1
Uridine	6	4.1205	87.126	4.113	0.007	86.923	0.20	1
Uridine	7	3.9084	63.6284	3.896	0.012	63.508	0.12	1
Uridine	8	3.8	63.6284	3.896	0.096	63.508	0.12	0
Uridine 5'-monophosphate	1	8.0837	144.7537	8.006	0.078	144.877	0.12	1
Uridine 5'-monophosphate	2	5.9853	90.951	5.981	0.004	91.168	0.22	1
Uridine 5'-monophosphate	3	5.9792	105.2944	5.961	0.018	105.51	0.22	1
Uridine 5'-monophosphate	4	4.411	76.6485	4.408	0.003	76.387	0.26	1
Uridine 5'-monophosphate	5	4.3423	72.7462	4.309	0.033	72.581	0.17	1
Uridine 5'-monophosphate	6	4.2529	86.6691	4.211	0.042	86.777	0.11	1
Uridine 5'-monophosphate	7	4.0177	65.857	ND		ND		0
Uridine 5'-monophosphate	8	3.9711	65.857	ND		ND		0
Uridine 5'-diphosphate	1	7.9871	144.3974	7.964	0.023	144.438	0.04	1
Uridine 5'-diphosphate	2	5.9652	105.2318	5.961	0.004	105.51	0.28	1
Uridine 5'-diphosphate	3	5.9594	91.2927	5.981	0.022	91.314	0.02	1
Uridine 5'-diphosphate	4	4.4226	71.8226	ND		ND		0
Uridine 5'-diphosphate	5	4.3827	76.4968	4.349	0.034	76.533	0.04	1
Uridine 5'-diphosphate	6	4.263	85.808	4.27	0.007	86.192	0.38	1
Uridine 5'-diphosphate	7	4.2173	66.7243	ND		ND		0
Guanosine monophosphate	1	8.1966	140.2158	ND		ND		0
Guanosine monophosphate	2	5.926	89.3628	5.941	0.015	89.558	0.20	1
Guanosine monophosphate	3	4.7423	76.7923	4.742	0.000	76.24	0.55	0
Guanosine monophosphate	4	4.4768	73.4174	4.486	0.009	73.313	0.10	1
Guanosine monophosphate	5	4.3154	87.2596	ND		ND		0
Guanosine monophosphate	6	3.9818	66.1295	ND		ND		0
Adenosine monophosphate	1	8.5422	142.61	ND		ND		0
Adenosine monophosphate	2	8.1599	155.2645	ND		ND		0
Adenosine monophosphate	3	6.0952	89.5866	6.138	0.043	89.997	0.41	1
Adenosine monophosphate	4	4.7749	77.2747	ND		ND		0

Adenosine monophosphate	5	4.513	73.2829	4.486	0.027	73.313	0.03	1
Adenosine monophosphate	6	4.3718	87.1412	4.388	0.016	86.923	0.22	1
Adenosine monophosphate	7	4.0377	66.2032	4.034	0.004	66.289	0.09	1
Adenosine diphosphate	1	8.5408	140.4289	ND		ND		0
Adenosine diphosphate	2	8.2974	148.3898	8.222	0.075	148.243	0.15	1
Adenosine diphosphate	3	5.9425	87.115	ND		ND		
Adenosine diphosphate	4	4.5752	73.7066	4.585	0.010	73.46	0.25	1
Adenosine diphosphate	5	4.2228	70.0585	4.191	0.032	70.24	0.18	1
Adenosine diphosphate	6	4.1437	65.406	4.113	0.031	65.264	0.14	1
Adenosine diphosphate	7	4.1173	83.3287	4.113	0.004	83.411	0.08	1
Adenosine diphosphate	8	4.0793	65.4112	4.093	0.014	65.118	0.29	1
Adenosine triphosphate	1	8.5457	142.4994	ND		ND		0
Adenosine triphosphate	2	8.2414	155.3692	8.242	0.001	155.121	0.25	1
Adenosine triphosphate	3	6.1476	89.2792	ND		ND		0
Adenosine triphosphate	4	4.8198	76.9573	ND		ND		0
Adenosine triphosphate	5	4.6445	72.9634	4.644	0.000	72.435	0.53	0
Adenosine triphosphate	6	4.423	86.6093	ND		ND		0
Adenosine triphosphate	7	4.3107	67.786	ND		ND		0
Adenosine triphosphate	8	4.252	67.7121	4.25	0.002	67.898	0.19	1
NAD	1	9.3323	142.5136	ND		ND		0
NAD	2	9.1566	145.0726	ND		ND		0
NAD	3	8.8693	148.3929	8.832	0.037	148.828	0.44	1
NAD	4	8.7989	148.3929	8.832	0.033	148.828	0.44	1
NAD	5	8.4053	142.4201	ND		ND		0
NAD	6	8.1945	131.1386	ND		ND		0
NAD	7	8.1183	155.3617	ND		ND		0
NAD	8	6.0826	102.5917	ND		ND		0
NAD	9	6.0183	89.3063	ND		ND		0
NAD	10	4.7572	76.6879	4.742	0.015	76.24	0.45	1
NAD	11	4.5461	89.6301	ND		ND		0
NAD	12	4.5107	73.0308	4.486	0.025	73.313	0.28	1
NAD	13	4.4876	80.345	4.427	0.061	80.777	0.43	1

NAD	14	4.4346	73.3674	4.427	0.008	73.021	0.35	1
NAD	15	4.3764	86.4608	4.349	0.027	86.338	0.12	1
NAD	16	4.3642	67.5533	4.408	0.044	67.459	0.09	1
NAD	17	4.2357	67.5533	4.408	0.172	67.459	0.09	0
NAD	18	4.247	68.0556	4.25	0.003	68.045	0.01	1
NAD	19	4.2175	68.0556	4.25	0.032	68.045	0.01	1
NADP	1	8.18	131.3383	ND		ND		0
NADP	2	6.0997	89.1607	5.941	0.159	89.558	0.40	0
NADP	3	6.0309	102.7257	6.02	0.011	103.168	0.44	1
NADP	4	4.9861	78.8941	4.958	0.028	78.728	0.17	1
NADP	5	4.6174	72.7144	ND		ND		0
NADP	6	4.4965	89.7429	4.486	0.011	88.241	1.50	0
NADP	7	4.4542	80.4734	4.427	0.027	80.777	0.30	1
NADP	8	4.4059	73.4697	4.427	0.021	73.46	0.01	1
NADP	9	4.3726	85.7604	4.349	0.024	86.192	0.43	1
NADP	10	4.2552	67.7051	4.408	0.153	67.606	0.10	1
NADP	11	4.2467	68.1346	4.25	0.003	68.045	0.09	1
Quinic acid	1	4.1292	73.0325	4.132	0.003	73.313	0.28	1
Quinic acid	2	4.0061	69.718	ND		ND		0
Quinic acid	3	3.5372	77.8703	3.542	0.005	77.704	0.17	1
Quinic acid	4	2.0529	43.3275	2.068	0.015	43.605	0.28	1
Quinic acid	5	2.0353	40.1645	2.048	0.013	40.239	0.07	1
Quinic acid	6	1.948	40.1651	1.969	0.021	40.093	0.07	1
Quinic acid	7	1.8595	43.3272	1.871	0.012	43.605	0.28	1
t-Ferulic acid	1	7.3185	143.7394	7.2	0.119	142.535	1.20	0
t-Ferulic acid	2	7.247	113.7738	ND		ND		0
t-Ferulic acid	3	7.1179	124.8569	7.141	0.023	123.656	1.20	0
t-Ferulic acid	4	6.92	118.426	6.905	0.015	118.681	0.25	1
t-Ferulic acid	5	6.3813	124.5149	ND		ND		0
t-Ferulic acid	6	3.8921	58.7183	3.896	0.004	58.679	0.04	1
Mandelic acid	1	7.4202	129.7461	7.357	0.063	129.657	0.09	0
Mandelic acid	2	7.4202	131.4467	7.416	0.004	130.681	0.77	0

Mandelic acid	3	7.379	130.8475	7.357	0.022	129.657	1.19	0
Mandelic acid	4	4.977	77.7019	4.939	0.038	77.265	0.44	1
DL-Malic acid	1	4.289	73.2436	4.27	0.019	73.606	0.36	1
DL-Malic acid	2	2.6553	45.4634	2.638	0.017	45.8	0.34	1
DL-Malic acid	3	2.3431	45.4634	2.441	0.098	44.776	0.69	0
Citric acid	1	2.659	48.7114	2.599	0.060	48.581	0.13	0
Citric acid	2	2.5208	48.7114	2.599	0.078	48.727	0.02	0
Folic acid	1	8.4413	151.2733	ND		ND		0
Folic acid	2	7.4112	131.6746	7.416	0.005	132.145	0.47	1
Folic acid	3	6.2397	114.1605	ND		ND		0
Folic acid	4	4.2791	58.9113	ND		ND		0
Folic acid	5	4.0351	47.7659	ND		ND		0
Folic acid	6	2.3348	36.939	2.304	0.031	36.873	0.07	1
Folic acid	7	2.164	31.0478	2.127	0.037	31.458	0.41	1
Folic acid	8	2.0257	31.0478	1.989	0.037	31.751	0.70	0
2-Oxoglutaric acid	1	2.9974	38.6303	ND		ND		0
3-Oxoglutaric acid	2	2.423	33.4233	2.441	0.018	33.507	0.08	1
GABA	1	3.01	42.2135	3.012	0.002	41.995	0.22	1
GABA	2	2.2803	37.0498	2.304	0.024	36.873	0.18	1
GABA	3	1.8911	26.3781	1.891	0.000	26.629	0.25	1
Chlorogenic acid	1	7.64	148.7617	7.593	0.047	148.535	0.23	1
Chlorogenic acid	2	7.5531	148.7389	7.593	0.040	148.535	0.20	1
Chlorogenic acid	4	7.089	125.3337	6.944	0.145	125.559	0.23	0
Chlorogenic acid	5	6.9179	119.0127	6.925	0.007	118.973	0.04	1
Chlorogenic acid	3	7.1536	117.8173	7.2	0.046	117.803	0.01	1
Chlorogenic acid	6	6.3504	117.274	6.256	0.094	117.071	0.20	0
Chlorogenic acid	9	3.8664	75.6838	3.837	0.029	75.655	0.03	1
Chlorogenic acid	7	5.3145	73.7767	ND		ND		0
Chlorogenic acid	8	4.246	73.5159	4.27	0.024	73.752	0.24	1
Chlorogenic acid	13	2.0149	41.15	2.166	0.151	41.263	0.11	0
Chlorogenic acid	10	2.1859	41.141	2.166	0.020	41.263	0.12	1
Chlorogenic acid	11	2.1329	40.0724	2.127	0.006	40.093	0.02	1

Chlorogenic acid	12	2.0279	40.0724	2.028	0.000	40.093	0.02	1
Threonic acid	1	4.056	75.1363	4.054	0.002	74.923	0.21	1
Threonic acid	2	3.9829	75.5457	3.975	0.008	75.655	0.11	1
Threonic acid	3	3.6917	65.6394	3.66	0.032	65.441	0.20	1
Threonic acid	3	3.6294	65.6394	3.66	0.031	65.441	0.20	1
L-Arginine	1	3.7614	57.2627	3.778	0.017	57.215	0.05	1
L-Arginine	2	3.2354	43.3232	3.228	0.007	43.312	0.01	1
L-Arginine	3	1.9062	30.4926	1.91	0.004	30.434	0.06	1
L-Arginine	4	1.6799	26.4533	1.733	0.053	26.775	0.32	1
L-Asparagine	1	3.9929	54.1301	4.014	0.021	54.142	0.01	1
L-Asparagine	2	2.9439	37.4319	2.953	0.009	37.312	0.12	1
L-Asparagine	3	2.8433	37.3557	2.874	0.031	37.312	0.04	1
L-Aspartic acid	1	3.9079	55.0859	3.896	0.012	55.02	0.07	1
L-Aspartic acid	2	2.8426	39.3943	2.815	0.028	39.361	0.03	1
L-Aspartic acid	3	2.8031	39.4769	2.815	0.012	39.361	0.12	1
L-Aspartic acid	4	2.7119	39.3327	2.815	0.103	39.361	0.03	0
L-Aspartic acid	5	2.6896	39.3327	2.697	0.007	39.361	0.03	1
L-Aspartic acid	6	2.6675	39.341	2.697	0.030	39.361	0.02	1
L-Aspartic acid	7	2.6455	39.3493	2.697	0.051	39.361	0.01	1
L-Cysteine	1	3.9488	58.8817	3.955	0.006	59.264	0.38	1
L-Cysteine	2	3.0517	27.7941	3.13	0.078	27.653	0.14	0
L-Glutamic acid	1	3.7425	57.6427	3.739	0.004	57.508	0.13	1
L-Glutamic acid	2	2.34	36.356	2.304	0.036	36.873	0.52	1
L-Glutamic acid	3	2.085	29.8225	2.087	0.002	29.702	0.12	1
L-Glutamine	1	3.7577	57.232	3.739	0.019	57.508	0.28	1
L-Glutamine	2	2.4393	33.9285	2.461	0.022	33.653	0.28	1
L-Glutamine	3	2.1223	29.2856	2.127	0.005	29.117	0.17	1
L-Histidine	1	8.0254	138.3663	ND		ND		0
L-Histidine	2	7.1359	119.9937	7.102	0.034	119.998	0.00	1
L-Histidine	3	4.0037	57.2847	ND		ND		0
L-Histidine	4	3.2941	30.2042	3.248	0.046	30.434	0.23	1
L-Histidine	5	3.2668	30.2042	3.248	0.019	30.434	0.23	1

L-Histidine	6	3.2076	30.0802	3.248	0.040	30.434	0.35	1
L-Leucine	1	3.7414	56.2106	3.719	0.022	56.337	0.13	1
L-Leucine	2	1.7026	42.5958	1.694	0.009	42.58	0.02	1
L-Leucine	3	0.95	24.3632	0.947	0.003	24.872	0.51	1
L-Lysine	1	3.7466	57.4527	3.739	0.008	57.508	0.06	1
L-Lysine	2	3.0175	42.1193	3.012	0.006	42.141	0.02	1
L-Lysine	3	1.8841	32.6543	1.812	0.072	32.214	0.44	1
L-Lysine	4	1.7181	29.1523	1.714	0.004	29.263	0.11	1
L-Lysine	5	1.4925	24.0412	1.478	0.015	24.287	0.25	1
L-Lysine	6	1.43	24.0412	1.439	0.009	24.287	0.25	1
L-Phenylalanine	1	7.419	131.8113	7.416	0.003	132.145	0.33	1
L-Phenylalanine	2	7.3658	130.428	ND		ND		0
L-Phenylalanine	3	7.3155	132.1171	7.318	0.002	132.145	0.03	1
L-Phenylalanine	4	3.9758	58.933	3.995	0.019	59.118	0.19	1
L-Phenylalanine	5	3.2738	39.1507	3.287	0.013	39.214	0.06	1
L-Phenylalanine	6	3.1095	39.1507	3.13	0.020	39.214	0.06	1
Proline	1	4.1272	64.0393	4.132	0.005	63.654	0.39	1
Proline	2	3.4072	48.9572	3.425	0.018	49.02	0.06	1
Proline	3	3.3235	48.9572	3.326	0.002	49.02	0.06	1
Proline	4	2.344	31.7204	2.343	0.001	31.751	0.03	1
Proline	5	2.0678	31.8401	2.028	0.040	32.483	0.64	0
Proline	6	1.9924	26.4536	1.969	0.023	26.19	0.26	1
L-Serine	1	3.9545	63.0808	3.995	0.041	62.776	0.30	1
L-Serine	2	3.8325	59.1777	3.837	0.005	59.264	0.09	1
Thiamin	1	9.423	156.5705	ND		ND		0
Thiamin	2	8.0471	159.717	ND		ND		0
Thiamin	3	5.4486	53.8722	ND		ND		0
Thiamin	4	3.8854	63.2112	3.896	0.011	63.362	0.15	1
Thiamin	5	3.1842	31.8779	ND		ND		0
Thiamin	6	2.5658	13.7795	2.559	0.007	13.457	0.32	1
Thiamin	7	2.4896	26.7363	2.461	0.029	26.336	0.40	1
L-Tyrosine	1	7.194	133.4862	7.192	0.002	133.756	0.27	1

L-Tyrosine	2	6.8916	118.8917	6.895	0.003	118.681	0.21	1
L-Tyrosine	3	3.9329	58.9873	3.955	0.022	59.118	0.13	1
L-Tyrosine	4	3.1749	38.2714	3.13	0.045	38.487	0.22	1
L-Tyrosine	5	3.0695	38.2714	3.051	0.019	38.19	0.08	1
DL-Arabinose	1	4.502	99.5999	4.565	0.063	99.502	0.10	0
DL-Arabinose	2	5.2295	95.3407	5.293	0.064	95.558	0.22	0
DL-Arabinose	3	3.4964	74.7927	3.523	0.027	74.338	0.45	1
DL-Arabinose	4	3.9294	71.2621	3.916	0.013	71.411	0.15	1
DL-Arabinose	5	3.8082	71.3042	3.837	0.029	71.411	0.11	1
DL-Arabinose	6	3.651	75.234	3.621	0.030	75.216	0.02	1
DL-Arabinose	7	3.8874	69.0766	ND		ND		0
DL-Arabinose	8	3.8676	71.4092	3.837	0.031	71.411	0.00	1
DL-Arabinose	9	3.6671	69.2026	ND		ND		0
DL-Arabinose	10	4.0135	65.2098	ND		ND		0
DL-Arabinose	11	3.635	65.2308	3.66	0.025	65.264	0.03	1
DL-Arabinose	12	3.995	71.5353	3.837	0.158	71.411	0.12	1
D-Galactose	1	5.255	95.0068	5.234	0.021	95.119	0.11	1
D-Galactose	2	4.5733	99.2612	4.565	0.008	99.509	0.25	1
D-Galactose	3	4.0732	73.1943	4.034	0.039	73.46	0.27	1
D-Galactose	4	3.9795	72.1345	3.995	0.016	72.289	0.15	1
D-Galactose	5	3.9221	71.5533	3.837	0.085	71.411	0.14	0
D-Galactose	6	3.8476	72.0661	3.837	0.011	72.581	0.51	0
D-Galactose	7	3.7965	71.2114	3.837	0.041	71.411	0.20	1
D-Galactose	8	3.7369	63.827	3.7	0.037	63.654	0.17	1
D-Galactose	9	3.6965	77.8437	3.66	0.036	77.411	0.43	1
D-Galactose	10	3.6412	75.5873	ND		ND		0
D-Galactose	11	3.4773	74.7327	ND		ND		0
D-Glucose	1	5.2213	94.8809	5.234	0.013	95.119	0.24	1
D-Glucose	2	3.8891	63.3933	3.896	0.007	63.654	0.26	1
D-Glucose	3	3.8219	63.3242	3.837	0.015	63.508	0.18	1
D-Glucose	4	3.8126	74.1491	3.818	0.005	74.338	0.19	1
D-Glucose	5	3.7014	75.6017	3.7	0.001	75.655	0.05	1

D-Glucose	6	3.72	63.5317	3.719	0.001	63.508	0.02	1
D-Glucose	7	3.5208	74.322	3.523	0.002	74.338	0.02	1
D-Glucose	8	3.4582	78.6451	3.464	0.006	78.728	0.08	1
D-Glucose	9	3.398	72.4545	3.385	0.013	72.435	0.02	1
D-Glucose	10	3.2266	77.0196	3.228	0.001	77.118	0.10	1
D-Mannose	1	3.9245	73.6845	3.964	0.039	73.313	0.37	1
D-Mannose	2	3.8379	73.1236	3.857	0.019	73.469	0.35	1
D-Mannose	3	3.8757	63.8181	3.896	0.020	63.654	0.16	1
D-Mannose	4	3.7492	63.7851	3.759	0.010	63.362	0.42	1
D-Mannose	5	3.8002	75.2684	3.818	0.018	75.655	0.39	1
D-Mannose	6	3.6516	69.7248	3.641	0.011	69.801	0.08	1
D-Mannose	7	3.5673	69.4938	3.562	0.005	69.655	0.16	1
D-Mannose	8	3.3721	78.9312	ND		ND		0
D-Rhamnose	1	5.1056	96.7613	4.899	0.207	96.582	0.18	0
D-Rhamnose	2	4.8585	96.2135	5.175	0.317	96.875	0.66	0
D-Rhamnose	3	3.5966	75.4967	ND		ND		0
D-Rhamnose	4	3.4367	74.8493	3.464	0.027	74.923	0.07	1
D-Rhamnose	5	3.3756	74.6003	3.346	0.030	74.923	0.32	1
D-Rhamnose	6	3.9193	73.7537	3.964	0.045	73.752	0.00	1
D-Rhamnose	7	3.7943	72.7079	3.837	0.043	72.581	0.13	1
D-Rhamnose	8	3.8496	70.9151	3.837	0.013	71.118	0.20	1
D-Rhamnose	9	1.2715	19.4134	1.281	0.009	19.604	0.19	1
D-Xylose	1	5.1831	94.978	ND		ND		0
D-Xylose	2	4.5752	99.4278	4.565	0.010	99.509	0.08	1
D-Xylose	3	4.555	99.4355	4.565	0.010	99.509	0.07	1
D-Xylose	4	3.9372	67.9133	3.936	0.001	68.338	0.42	1
D-Xylose	5	3.8939	67.8796	3.936	0.042	68.338	0.46	1
D-Xylose	6	3.6871	63.6465	3.7	0.013	63.654	0.01	1
D-Xylose	7	3.6655	63.6355	3.7	0.035	63.654	0.02	1
D-Xylose	8	3.6782	72.121	3.621	0.057	72.435	0.31	0
D-Xylose	9	3.6534	75.5472	ND		ND		0
D-Xylose	10	3.6286	75.6704	ND		ND		0

D-Xylose	11	3.6229	72.1457	3.523	0.100	72.435	0.29	0
D-Xylose	12	3.6047	72.0717	3.621	0.016	72.435	0.36	1
D-Xylose	13	3.5837	72.1457	3.523	0.061	72.435	0.29	0
D-Xylose	14	3.5239	74.2797	3.523	0.001	74.338	0.06	1
D-Xylose	15	3.4976	74.2447	3.523	0.025	74.338	0.09	1
D-Xylose	16	3.4207	78.6697	3.385	0.036	78.728	0.06	1
D-Xylose	17	3.3061	67.8678	3.307	0.001	68.045	0.18	1
D-Xylose	18	3.2144	76.9117	3.228	0.014	77.118	0.21	1
D-Ribose	1	5.388	99.0368	ND		ND		0
D-Ribose	2	5.2549	103.8029	ND		ND		0
D-Ribose	3	4.9273	96.5326	4.978	0.051	96.729	0.20	1
D-Ribose	4	4.2111	73.1063	ND		ND		0
D-Ribose	5	4.1355	85.8696	ND		ND		0
D-Ribose	6	4.1139	73.4294	4.132	0.018	73.313	0.12	1
D-Ribose	7	4.1103	71.733	ND		ND		0
D-Ribose	8	4.0059	77.9531	ND		ND		0
D-Ribose	9	3.9735	85.0618	ND		ND		0
D-Ribose	10	3.9268	71.8138	3.936	0.009	71.411	0.40	1
D-Ribose	11	3.8836	69.8751	3.837	0.047	69.655	0.22	1
D-Ribose	12	3.8332	65.6745	3.798	0.035	65.264	0.41	1
D-Ribose	13	3.8332	72.6216	3.798	0.035	72.435	0.19	1
D-Ribose	14	3.6892	65.6745	3.66	0.029	65.264	0.41	1
D-Ribose	15	3.5308	73.6718	3.464	0.067	73.46	0.21	0
D-Ribose	16	4.0975	72.6136	4.073	0.024	72.728	0.11	1
D-Ribose	17	3.8635	69.8978	3.837	0.027	69.655	0.24	1
D-Ribose	18	3.932	65.9066	3.916	0.016	65.557	0.35	1
D-Ribose	19	3.8032	65.2472	3.798	0.005	65.264	0.02	1
D-Ribose	20	3.7336	64.0672	3.641	0.093	64.24	0.17	0
D-Ribose	21	3.6582	65.2472	3.66	0.002	65.264	0.02	1
D-Ribose	22	3.6536	64.0672	3.641	0.013	64.24	0.17	1
D-Ribose	23	3.6026	65.9066	ND		ND		0
D-Fructose	1	4.1055	78.0741	4.113	0.008	78.289	0.21	1

D-Fructose	2	4.1057	77.4277	4.113	0.007	77.265	0.16	1
D-Fructose	3	4.0141	66.12	4.014	0.000	66.289	0.17	1
D-Fructose	4	3.9871	72.1456	3.995	0.008	72.142	0.00	1
D-Fructose	5	3.8856	72.5344	3.877	0.009	72.435	0.10	1
D-Fructose	6	3.8189	83.5814	3.818	0.001	83.411	0.17	1
D-Fructose	7	3.8009	65.3592	3.798	0.003	65.264	0.10	1
D-Fructose	8	3.7858	70.4286	3.778	0.008	70.386	0.04	1
D-Fructose	9	3.6995	66.4439	3.7	0.001	66.289	0.15	1
D-Fructose	10	3.6659	65.5982	3.542	0.124	66.728	1.13	1
D-Fructose	11	3.5895	65.5982	3.562	0.028	65.411	0.19	1
D-Fructose	12	3.5423	65.419	3.562	0.020	65.411	0.01	1
D-Fructose	13	3.5523	66.8327	3.542	0.010	66.728	0.10	1
D-Xylulose	1	4.3701	77.5022	4.309	0.061	77.265	0.24	0
D-Xylulose	2	4.1821	72.3718	4.211	0.029	72.581	0.21	1
D-Xylulose	3	4.038	78.8612	3.995	0.043	78.874	0.01	1
D-Xylulose	4	3.6298	72.3718	3.621	0.009	72.581	0.21	1
D-Xylulose	5	3.6015	65.7333	3.66	0.059	65.264	0.47	0
D-Xylulose	6	3.5719	65.7333	3.66	0.088	65.264	0.47	1
Maltose	1	5.3982	102.2808	ND		ND		0
Maltose	2	5.2223	94.6453	5.234	0.012	94.826	0.18	1
Maltose	3	4.6441	98.5034	4.644	0.000	98.631	0.13	1
Maltose	4	3.963	75.9677	ND		ND		0
Maltose	5	3.9273	72.6928	3.896	0.031	72.581	0.11	1
Maltose	6	3.9031	63.2344	3.896	0.007	63.508	0.27	1
Maltose	7	3.8098	63.2961	3.818	0.008	63.508	0.21	1
Maltose	8	3.763	63.2852	3.759	0.004	63.508	0.22	1
Maltose	9	3.7686	63.2892	3.759	0.010	63.508	0.22	1
Maltose	10	3.7599	79.0088	3.719	0.041	78.874	0.13	1
Maltose	11	3.7105	75.4414	3.7	0.011	74.338	1.10	1
Maltose	12	3.6735	75.6753	ND		ND		0
Maltose	13	3.6296	79.4181	3.621	0.009	79.606	0.19	1
Maltose	14	3.5829	77.2543	ND		ND		0

Maltose	15	3.5706	74.3887	3.523	0.048	74.484	0.10	1
Maltose	16	3.4114	72.1372	3.385	0.026	72.435	0.30	1
Maltose	17	3.2659	76.7572	3.228	0.038	76.972	0.21	1
Sucrose	1	5.3993	94.7981	5.411	0.012	94.826	0.03	1
Sucrose	2	3.8758	84.1442	3.818	0.058	84.289	0.14	0
Sucrose	3	4.2052	79.2158	4.211	0.006	78.874	0.34	1
Sucrose	4	4.0353	76.7517	4.034	0.001	76.826	0.07	1
Sucrose	5	3.8295	75.1572	3.877	0.047	75.069	0.09	1
Sucrose	6	3.742	75.3022	3.7	0.042	75.655	0.35	1
Sucrose	7	3.5464	73.7802	3.523	0.023	74.191	0.41	1
Sucrose	8	3.4743	71.9683	3.385	0.089	72.435	0.47	1
Sucrose	9	3.6647	64.0684	3.66	0.005	64.093	0.02	1
Sucrose	10	3.8089	62.7639	ND		ND		0
Sucrose	11	3.8091	65.0452	3.798	0.011	65.118	0.07	1
Cellobiose	1	5.2191	94.5353	5.175	0.044	93.655	0.88	1
Cellobiose	2	4.6535	98.4467	4.644	0.010	98.631	0.18	1
Cellobiose	3	4.4981	105.1863	ND		ND		0
Cellobiose	4	3.9498	62.6313	3.896	0.054	63.508	0.88	0
Cellobiose	5	3.9349	72.7171	3.896	0.039	72.435	0.28	1
Cellobiose	6	3.9213	63.285	3.896	0.025	63.508	0.22	1
Cellobiose	7	3.904	63.285	3.896	0.008	63.508	0.22	1
Cellobiose	8	3.8668	62.5379	3.877	0.010	62.484	0.05	0
Cellobiose	9	3.8136	74.0245	3.837	0.023	74.338	0.31	1
Cellobiose	10	3.8049	62.787	3.837	0.032	63.362	0.58	0
Cellobiose	11	3.7331	63.3162	3.719	0.014	63.508	0.19	1
Cellobiose	12	3.6365	81.2775	ND		ND		0
Cellobiose	13	3.6217	77.044	3.601	0.021	77.704	0.66	0
Cellobiose	14	3.5895	77.3864	3.601	0.011	77.704	0.32	1
Cellobiose	15	3.5697	73.9311	3.601	0.031	73.899	0.03	1
Cellobiose	16	3.5041	78.258	3.464	0.040	78.728	0.47	1
Cellobiose	17	3.4768	78.6005	3.464	0.013	78.728	0.13	1
Cellobiose	18	3.4124	72.219	3.385	0.027	72.435	0.22	1

Cellobiose	19	3.3134	75.8611	3.267	0.046	75.069	0.79	0
Cellobiose	20	3.275	76.6082	ND		ND		0
Raffinose	1	3.8181	71.043	ND		ND		0
Raffinose	2	5.4136	94.6172	5.411	0.003	94.973	0.36	1
Raffinose	3	3.6658	64.0701	3.7	0.034	63.654	0.42	1
Raffinose	4	3.6778	68.3888	3.68	0.002	68.191	0.20	1
Raffinose	5	4.9823	101.0836	ND		ND		0
Raffinose	6	3.9445	73.6971	4.034	0.089	73.46	0.24	0
Raffinose	7	4.0295	68.388	4.014	0.015	68.045	0.34	1
Raffinose	8	3.5359	72.0362	3.523	0.013	72.435	0.40	1
Raffinose	9	3.8299	65.0651	3.798	0.032	65.264	0.20	1
Raffinose	10	3.8866	72.0361	3.877	0.010	72.435	0.40	1
Raffinose	11	4.0409	74.0258	ND		ND		0
Raffinose	12	3.5601	73.6961	3.464	0.096	73.46	0.24	0
Raffinose	13	3.7537	75.358	3.719	0.035	75.655	0.30	1
Raffinose	14	3.7387	63.7411	3.7	0.039	63.654	0.09	1
Raffinose	15	3.7773	65.0645	3.66	0.117	65.264	0.20	0
Raffinose	16	3.9943	71.7113	ND		ND		0
Raffinose	17	4.217	79.0091	4.211	0.006	78.874	0.14	1
Raffinose	18	3.8888	83.9885	ND		ND		0
Raffinose	19	4.0528	76.6836	4.054	0.001	76.679	0.00	1
Glucuronic acid	1	5.2338	94.9997	5.234	0.000	94.826	0.17	1
Glucuronic acid	2	4.6311	98.7386	4.644	0.013	98.631	0.11	1
Glucuronic acid	3	4.0673	74.6321	4.073	0.006	74.338	0.29	1
Glucuronic acid	4	3.7134	79.0106	3.719	0.006	78.874	0.14	1
Glucuronic acid	5	3.7159	75.4552	3.719	0.003	75.655	0.20	1
Glucuronic acid	6	3.5654	74.185	3.542	0.023	74.338	0.15	1
Glucuronic acid	7	3.4996	74.8075	3.542	0.042	74.338	0.47	1
Glucuronic acid	8	3.4989	78.4636	3.483	0.016	78.874	0.41	1
Glucuronic acid	9	3.2748	76.9355	3.228	0.047	77.118	0.18	1
D-Sorbitol	1	3.8381	75.772	3.837	0.001	75.665	0.11	1
D-Sorbitol	2	3.8381	72.495	3.837	0.001	72.581	0.09	1

D-Sorbitol	3	3.8255	65.5621	3.798	0.027	65.264	0.30	1
D-Sorbitol	4	3.7664	73.788	3.778	0.012	73.46	0.33	1
D-Sorbitol	5	3.7312	65.2277	ND		ND		0
D-Sorbitol	6	3.6508	65.5398	3.66	0.009	65.264	0.28	1
D-Sorbitol	7	3.6458	73.8326	ND		ND		0
D-Sorbitol	8	3.6231	65.1831	3.66	0.037	65.264	0.08	1
D-Mannitol	1	3.863	65.9908	3.798	0.065	65.264	0.73	0
D-Mannitol	2	3.7976	71.9574	3.778	0.020	72.435	0.48	1
D-Mannitol	3	3.7597	73.4898	3.778	0.018	73.46	0.03	1
D-Mannitol	4	3.6861	65.9908	3.66	0.026	65.264	0.73	0
L-Iditol	1	3.8454	74.3645	3.837	0.008	74.338	0.03	1
L-Iditol	2	3.7305	73.7063	3.778	0.047	73.46	0.25	1
L-Iditol	3	3.7069	65.4042	3.66	0.047	65.118	0.29	1
L-Iditol	4	3.6547	65.4042	3.66	0.005	65.118	0.29	1
Trigonelline	1	9.166	148.397	9.127	0.039	148.682	0.28	1
Trigonelline	2	9.0736	148.397	8.97	0.104	148.974	0.58	0
Trigonelline	3	8.8799	148.7102	8.832	0.048	148.974	0.26	1
Trigonelline	4	8.7804	148.7102	8.832	0.052	148.974	0.26	1
Trigonelline	5	8.0772	130.3871	8.085	0.008	130.681	0.29	1
Trigonelline	6	4.437	51.0584	4.427	0.010	51.969	0.91	0
Trigonelline	7	8.8768	147.5165	8.832	0.045	147.657	0.14	1
Trigonelline	8	8.8	147.5843	8.832	0.032	147.657	0.07	1

Supplemental Table 5 Effect of the production systems on the canonical correlations between mineral nutrient (B, Mg, P, K, Ca, Mn, Fe, Zn) contents and the intensity of ^{1}H NMR signals. The values represent p values determined by Tukey's HSD test.

A) Organic and Amino acids	Organic fertilizers	Organic fertilizers + Pesticides	Chemical fertilizers	Chemical fertilizers + Pesticides
Organic fertilizers	1.000	0.563	0.333	0.089
Organic fertilizers + Pesticides	0.563	1.000	0.981	0.002
Chemical fertilizers	0.333	0.981	1.000	0.000
Chemical fertilizers + Pesticides	0.089	0.002	0.000	1.000

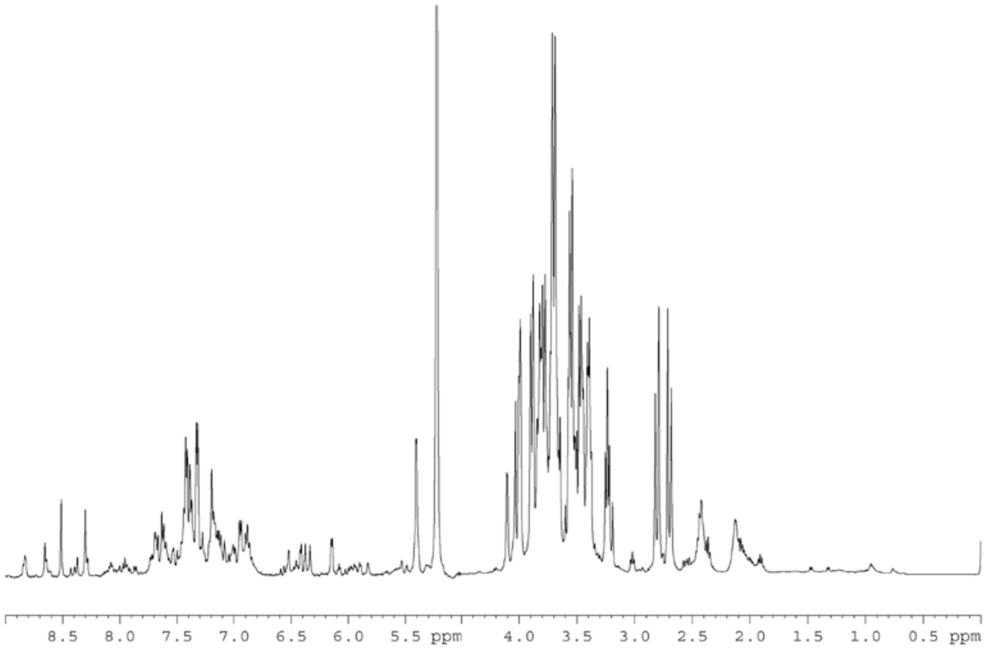
Organic and amino acid region is δ ~3.1 ppm.

B) Sugars	Organic fertilizers	Organic fertilizers + Pesticides	Chemical fertilizers	Chemical fertilizers + Pesticides
Organic fertilizers	1.000	0.083	0.016	0.819
Organic fertilizers + Pesticides	0.083	1.000	0.932	0.439
Chemical fertilizers	0.016	0.932	1.000	0.153
Chemical fertilizers + Pesticides	0.819	0.439	0.153	1.000

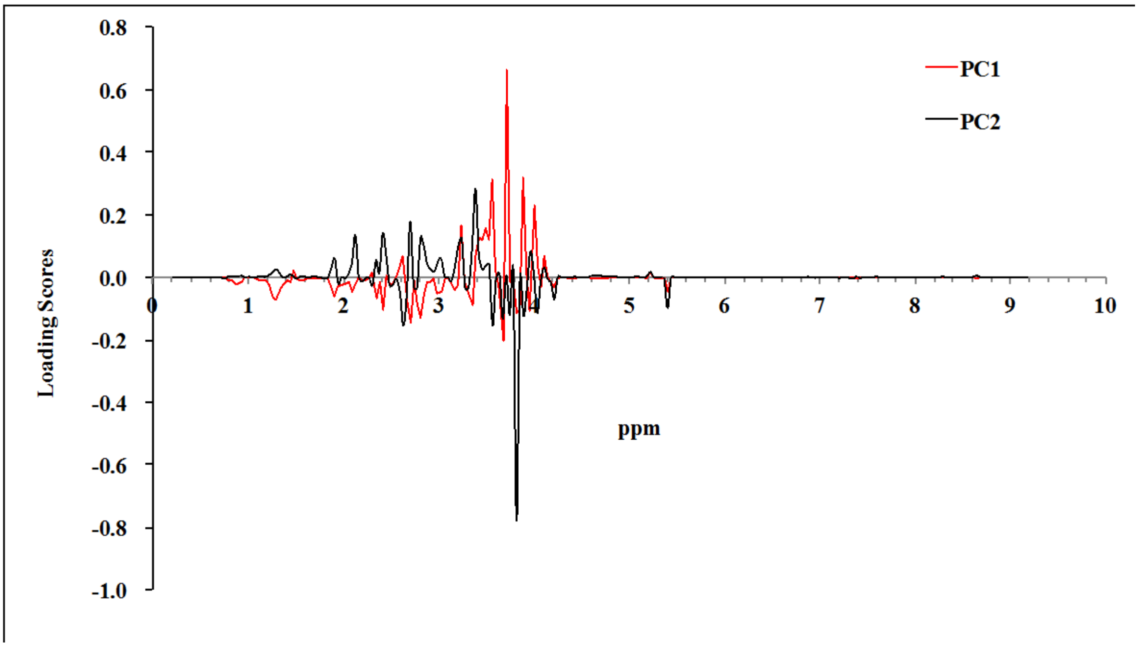
Sugar region is δ 3.1~5.2 ppm.

C) Aromatic substrates	Organic fertilizers	Organic fertilizers + Pesticides	Chemical fertilizers	Chemical fertilizers + Pesticides
Organic fertilizers	1.000	0.000	0.000	0.000
Organic fertilizers + Pesticides	0.000	1.000	0.000	0.000
Chemical fertilizers	0.000	0.000	1.000	0.998
Chemical fertilizers + Pesticides	0.000	0.000	0.998	1.000

Aromatic substrate region is δ 5.2~9.0 ppm.



Supplemental Figure 1 ^{1}H NMR spectrum of 70% methanol soluble metabolites in tomato fruits cultured with organic fertilizers.



Supplemental Figure 2　Principal component analysis loadings of soluble metabolites with the first principal component (PC1) and the second (PC2) in tomato fruits.