International Food Research Journal 22(6): 2288-2293 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Analyzing changes in metabolite profile during postharvest ripening in *Achras sapota* fruits: GC-MS based metabolomics approach

Das, S. and *De, B.

Phytochemistry and Pharmacognosy Research Laboratory, Department of Botany, University of Calcutta,35 Ballygunge Circular Road, Kolkata 700019 , India

Article history

Received: 10 December 2014
Received in revised form: 11 May 2015
Accepted: 21 May 2015

Keywords

Achras sapota
Fruit
Postharvest ripening
Metabolomics
GC-MS

Abstract

Achras sapota L. (syn. *Manilkara zapota* (L.) P. Royen) fruits are commonly known as sapodilla. The mature fruits when unripe are not edible. They are allowed to ripen at room temperature when the pulp softens and become edible. The aim of the present investigation was to determine the changes in metabolite composition during postharvest ripening over a span of 10 days following a GC-MS based metabolomics approach. The present metabolic data revealed, for the first time the composition of *A. sapota* fruit metabolites and changes in individual metabolite during postharvest ripening. Total 46 identified metabolites (11 sugars and sugar alcohols, 11 organic acids, 14 amino acids, 5 phenols, 4 fatty acids and 1 inorganic acid) and 20 tentatively identified compounds which showed significant differences during ripening could be detected. On the whole it appears that in spite of decrease in the level of many metabolites, a large number of amino acids and sugar alcohols increased in quantity ameliorating the fruit quality during ripening.

Introduction

Fruit quality is a direct function of metabolite content, and the compositional analysis of plant metabolites is an established application in metabolomics research (Moco *et al*., 2007). Organoleptic quality of fruit is a complex trait of fruit quality involving a combination of taste, flavor and aroma (Oms-oliu *et al*., 2007). The nutritional quality of fruits is correlated closely with the presence of soluble sugars, organic acids, essential fatty acids, amino acids and some major secondary metabolites. These compounds play an important role in balancing fruit quality and nutritional aspects. That is why fruit compositional analysis is of great interest to food chemists (Zhang *et al*., 2011). Fruit ripening generally influences the level of pigments (e.g. carotenoids and flavonoids), sugars, acids and aroma volatiles to make the fruit more appealing and promotes tissue softening (Giovannoni, 2004; Giovannoni, 2007).

Achras sapota L. [syn. *Manilkara zapota* (L.) P. Royen] commonly known as sapodilla, is one of the fruit crops of South Asia. The tree is native to Mexico and tropical America and is now cultivated throughout the tropics (Shui *et al*., 2004). The mature unripe fruits when harvested are not edible. They are allowed to ripen at room temperature when the pulp softens with change of color from greenish to brown and become edible. In our previous study, inhibitory activities of *A. sapota* fruit against α- glucosidase, α-amylase and angiotensin I- converting enzyme have been reported (Das *et al*., 2012; Das and De, 2013). The major objective of the present investigation was to monitor the metabolic changes during postharvest ripening. Changes in metabolite profile can be determined by a number of methods e.g. gas chromatography coupled with mass spectrometry (GC-MS), liquid chromatography coupled with mass spectrometry (LC-MS), nuclear magnetic resonance spectroscopy (NMR). NMR has a smaller dynamic range than mass based technologies for detection of metabolites (Sumner *et al*., 2003). A GC-MS based metabolomic approach was adopted to study the metabolic changes during the present study.

Metabolic changes during development of important fruits e.g. tomato (*Solanum lycopersicum* L.) (Carrari *et al*., 2006), strawberry (*Fraggaria* X *ananassa* Duch.) (Zhang *et al*., 2011), grape berry *(Vitis vinifera)* (Deluc *et al*., 2007), black raspberry (*Rubus coreanus* Miquel) (Kim *et al.*, 2011), guava (*Psidium guajava* L.) (Lee *et al*., 2010) have been reported. But there are very few reports regarding changes in metabolite pattern in fruits during postharvest ripening. Postharvest and pre harvest metabolic changes in peach (*Prunus persica* (L.) Stokes) (Lombardo *et al*., 2011) and tomato (Oms-oliu *et al.*, 2011) have been discussed. We report here changes in metabolite pattern during postharvest

*Corresponding author.
Email: *bratatide@hotmail.com*

ripening in sapodilla *(A. sapota)* fruit.

Materials and Methods

Plant material

Mature, unripe *Achras sapota* fruits (Variety cricket ball) of more or less equal sizes were collected right after their harvest in the morning from Agricultural Field Station of University of Calcutta, Baruipur, India during its peak fruiting season (i.e., the month of April, 2012). The surface of the fruits was cleaned with distilled water just after harvesting. These fruits were then stored at room temperature (day temperature 35°C ± 2; night temperature 25°C ± 1) in the open air from day 0 i.e. the day of harvesting, up to day 9.

Sample preparation

The fruits of *A. sapota* were peeled and the seeds were removed before extraction. The fruit flesh (60 ± 5 mg) (crushed to powder with liquid nitrogen) was extracted with 1 ml of methanol at 60°C for 15 min. Ribitol (20 µl of 200 µg ml^{-1}) was added as an internal standard. The extract was centrifuged at 14,000 rpm for 15 minutes. The supernatant was distributed into eppendorff tubes (4 x 50 µl) and evaporated to dryness. For each biological replication, four fruits were considered. The residue was derivatized with MSTFA [N-methyl-N-(trimethylsilyl)trifluoroacetamide] following the method of Roessner *et al.* (2006) for GC-MS analyses. The dried residues were re-dissolved in methoxamine hydrochloride in pyridine (30 mg/ml), shaken for 2 h at 37°C. Then MSTFA (40 µl) and retention time alkane mixture [prepared using n-dodecane, n-heptadecane, n-nonadecane, n-docosane, n-octacosane and n-hexatriacontane dissolved in tetrahydrofuran in concentration 0.03%] (10 µl) were added for derivatization.

GC-MS analysis

GC-MS analysis was carried out following the method of Roessner *et al.* (2006) after little modification. HP-5MS capillary column (Agilent J & W; GC Columns (USA) (length 30 m plus Duraguard 10 m, diameter 0.25 mm narrowbore, film 0.25 µm) was used. The analysis was performed under the following oven temperature programme: injection at 70ºC followed by 1 ºC min^{-1} oven temperature ramp to 76ºC and then by 6ºC min^{-1} to 300ºC and with 10 minute isothermal at 300ºC. Helium gas was used as the carrier gas at a flow rate of 1 ml / min (Carrier linear velocity 36.798 cm sec^{-1}). Samples (1 µl) were injected via the split mode onto the GC column. Automated mass spectral deconvolution and identification system (AMDIS) was used to deconvolute GC-MS results and to identify chromatographic peaks distinctly. Identification of the metabolites was carried out by comparing the fragmentation patterns of the mass spectra with entries of mass spectra library NIST 2008, Golm library and with Agilent Fiehn GC / MS Metabolomics library (2008) (Agilent Technologies Inc., Wilmington, USA). The presence of the metabolites was further confirmed by comparing the retention indices relative to n-alkanes (C_{12} – C_{36}) with those in Golm library. Retention times of some of the metabolites were also compared with that of the standards for confirmation of the metabolites.

Statistical analysis

The relative response ratios of all the metabolites were calculated after normalizing the peak areas of the compounds by sample fresh weight and by the peak area of the internal standard. Data were analyzed by one way ANOVA to describe the significance of variation of each metabolite level between days 0 to 9. p Values of the metabolites ≤ 0.05, were considered significant. Principal component analysis was carried out using SPSS software (Version 16).

Results and Discussions

The mature but unripe fruits, on the day of harvest, were greenish brown in colour with pale yellow flesh, very firm to touch. The flesh was firm and astringent in taste during day 0 and 1. From day 2 onwards the fruits started to ripen. At around days 2-3, the fruits became softer and the colour of the flesh changed to an orange shade, very sweet in taste with a characteristic flavor, perfect stage of ripening to consume this fruit. Flesh of over ripened fruits at around days 8-9 gradually turned from orange to brownish black, tasted sweeter, but with lesser aroma and not very pleasant in taste.

During our study a total of 46 metabolites of known structure comprising 11 sugars and sugar alcohols, 11 organic acids, 14 amino acids, 5 phenols, 4 fatty acids, 1 inorganic acid and 20 tentatively identified compounds, could be detected by GC-MS analysis of the MSTFA derivatized samples during postharvest ripening (Table 1). Relative response ratios of each metabolite were calculated. Such data for each identified metabolite was compared in Figure 1 and Figure 2. Relative response ratios of the metabolites are routinely described (Roessner *et al*., 2000) for semi quantitative comparison.

Sugars and sugar alcohols are one of the most important contributory determinants for fruit taste

Table 1. Metabolites detected in *A. sapota* fruit

Organic acids	Amino acids	Sugars	Phenols	Fatty acids
Citric acid	Alanine	Allose*	Arbutin*	Arachidic acid*
Fumaric acid	Asparagine*	Altrose*	Benzoic acid	4-Guanidinobutyric acid*
Galacturonic acid*	Aspartic acid	Galactose*	Catechin	Heptadecanoic acid*
Glucoheptonic acid*	Glutamic acid	Lactose	Chlorogenic acid	Linoleic acid
Gluconic acid	Glutamine	Melezitose	Gallic acid	Oleic acid
Glyceric acid	Glycine	Raffinose	Orcinol*	Palmitic acid
Glycolic Acid	Isoleucine	Sucrose	Quinic acid	Stearic acid
Imminodiacetic acid*	Lysine	Trehalose	Resorcinol*	
2-Keto-L-gulonic acid*	Norleucine*	Allo-inositol*		
Lactic acid	Ornithine	Arabitol*		
Lactobionic acid*	Proline	Galactinol		
Maleic acid	Pyroglutamic acid	Glycerol		
Malic acid	Serine	Mannitol		
Malonic acid	Threonine	Myo-inositol		
Oxalic acid	Tyrosine	Sorbitol		
Pipecolic acid*	Valine	Threitol*		
Succinic acid		Xylitol		
Inorganic acid				
Phosphoric acid				

*Tentatively identified metabolites on the basis of MS fragmentation pattern

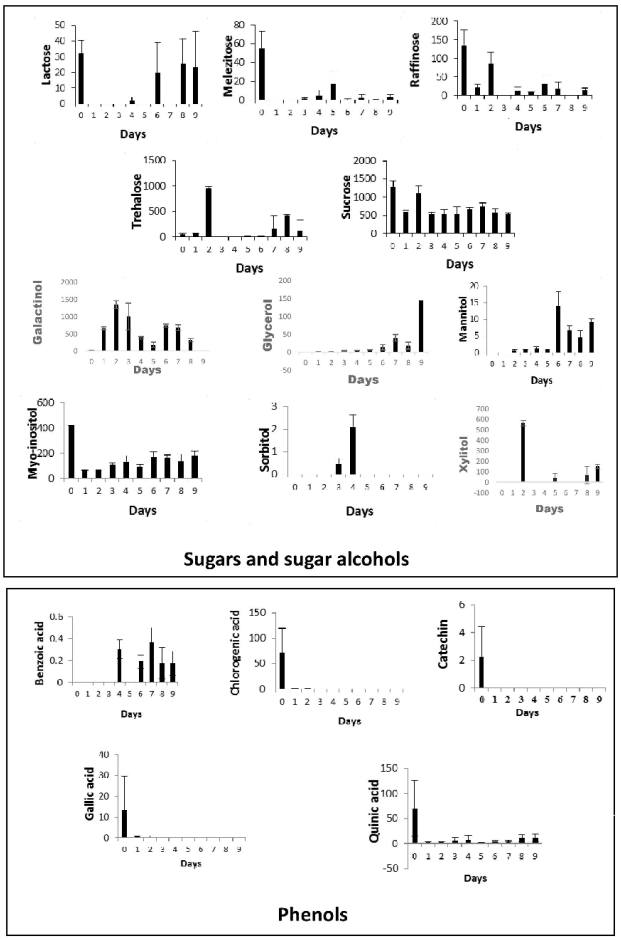

Figure 1. Identified metabolites (sugars, sugar alcohols and phenols) during postharvest ripening in *A. sapota.* Y axis represents relative response ratio (RRR) / g fresh weight. Data are mean values ± sd

(Zhang *et al.*, 2011). Relative response ratios of the sugars and sugar alcohols at different postharvest period are shown (Figure1). Significant decreases in the levels of lactose, melezitose, raffinose and sucrose were noticed. But sugar alcohols like galactinol, glycerol and mannitol were significantly higher during postharvest ripening over that of day 0. Myo-inositol, an important biosynthetic precursor of many cell wall polysaccharides (Oms-oliu *et al.*, 2007), decreased in content. The hydrogenated carbohydrates sugar alcohols (also known as polyols) are used as sugar replacers. They have potential health benefits due to low carcinogenicity, low glycaemia, low insulinaemia, low energy value, as source of substrate for healthy colon and intestinal tolerance (Livesey, 2003).

The phenolic compounds detected in the fruit flesh during postharvest ripening (Figure 1) revealed sharp decline in the concentrations of gallic acid (18 fold) and chlorogenic acid (57 fold) on day 1. The two compounds could not be detected from day 3 onwards. Quinic acid was the only phenolic compound detected right from day 0 to day 9 and a sharp decrease (20 fold) in this metabolite level was observed from day 0 to day 1. Benzoic acid, which could be detected first on day 4, was found in the ripe fruits up to day 9.

Organic acids are important components of fruits that strongly influence the fruit taste. During the study malic acid, lactic acid and succinic acid were detected as the major fruit acids, found at all stages of ripening. On the other hand citric acid, fumaric acid, gluconic acid and oxalic acid were detected only at certain stages of ripening. Maleic acid and glycolic acid were detected only in very unripe fruits (Figure 2). Significant changes in metabolite level of some organic acids were observed during post-harvest

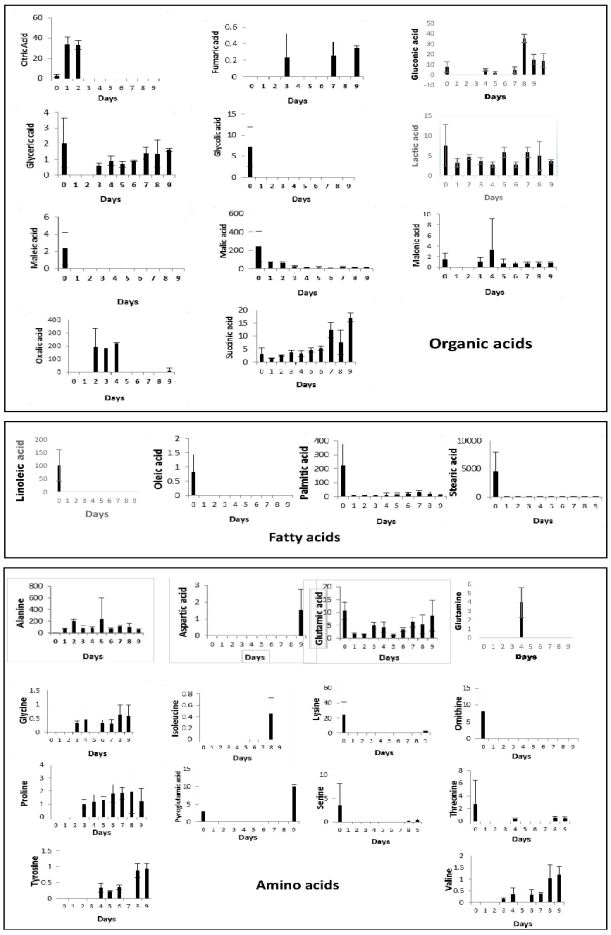

Figure 2. Identified metabolites (organic acids, fatty acids and amino acids) during postharvest ripening in *A. sapota*. Y axis represents relative response ratio (RRR) / g fresh weight. Data are mean values ± sd

maturation of this fruit. The tricarboxylic acid cycle metabolites detected were citric acid, succinic acid, fumaric acid and malic acid. There was significant increase of citric acid (13 fold) level on day 1. From day 1 to day 2 the content decreased 1.5 fold and was below the detection limit from day 3 onwards. Succinic acid on the other hand gradually increased with ripening. The levels of lactic acid and malic acid decreased significantly. Compound like gluconic acid, believed to be the intermediates of tartaric acid biosynthesis (De bolt *et al.*, 2006) increased during post-harvest ripening stages.

Fatty acids like palmitic acid and stearic acid were present in the fruit flesh at all the stages of ripening. There was a significant decrease in concentrations from day 0 to day 1 in cases of palmitic acid (23 fold) and stearic acid (243 fold). Linoleic acid and oleic acid appeared on the day 0 only (Figure 2).

The variation in the levels of amino acids with different stages of postharvest ripening of this fruit is shown in Figure 2. Glycine, derived from serine, could not be detected on first three days and then detected up to day 9 (except on day 5). Valine and isoleucine, the pyruvate derived amino acids,

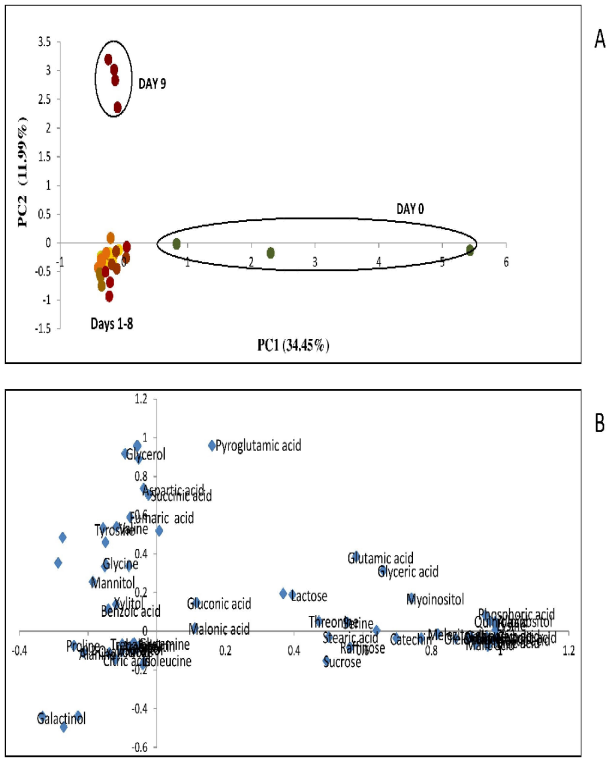

Figure 3. Multivariate analysis. A: PCA score plot; B: Loading plot showing the components

gradually increased up to day 9. Tricarboxylic acid cycle derived organic acids serve as the precursor of many amino acids. Aspartic acid (day 9) derived from oxaloacetic acid was detected during over ripened stages of the fruit. Proline, which could not be detected on days 0, 1 and 2, was present from day 3 onwards. The emerging evidences show that traditionally classified nonessential amino acids play important roles in gene expression, cell signaling and thus have effect on cell proliferation, differentiation, metabolism, homoeostasis and function (Haynes *et al.*, 2009; Stipanuk *et al.*, 2006; Wu, 2010). Both essential and nonessential amino acids should be present in diet to optimize health (Wu, 2010). Amongst the nonessential amino acids detected in *A. sapota* fruits, all (aspartate, glutamic acid, glycine and tyrosine) except serine increased in quantity with postharvest ripening. The essential amino acids lysine and threonine decreased whereas isoleucine and valine increased in quantity.

The data set obtained after the GC-MS analysis was subjected to Principal Component Analysis (PCA). Principal component 1 (PC1) and principal component 2 (PC2) score plot segregated three distinct groups separating day 0 (Group I) and day 9 (Group III) distinctly from the rest i.e. days 1-8 (Group II) (Figure 3A). The findings suggest that there is a metabolic change from day 0 to post harvest ripening stages. Loading plot (Figure 3B) analysis suggested that the major metabolites

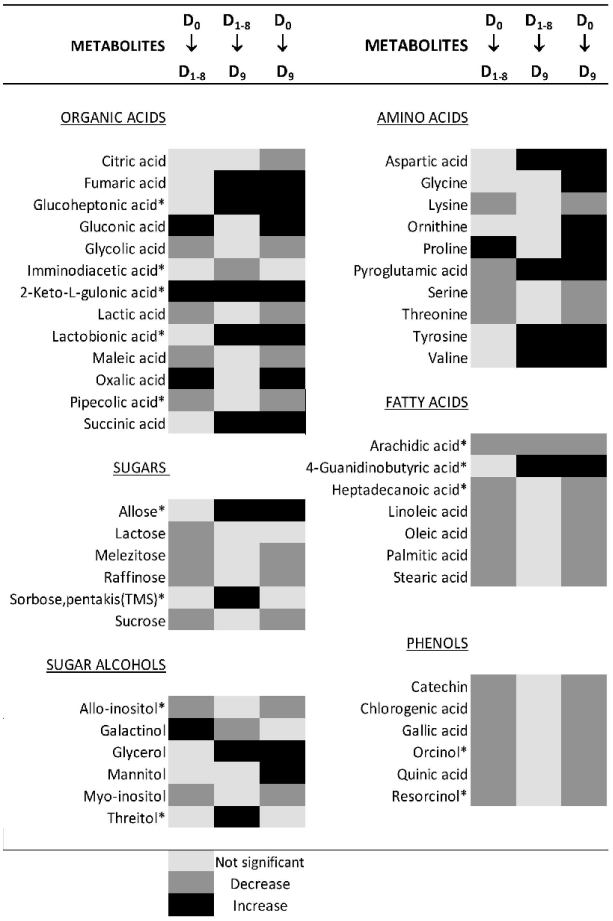

Figure 4. Identification of significant marker metabolites D_0: group I; D_{1-8}: Group II; D_9: Group III

responsible for such segregation of day 0 are aspartic acid, pyroglutamic acid, tyrosine, valine, proline, galactinol, glycerol, sucrose, raffinose, catechin etc. As a complement to estimate the marker metabolites, significant increase and decrease of metabolites are shown in Figure 4. In spite of decrease in the level of many metabolites, a large number of amino acids and sugar alcohols increased in quantity. The study revealed that metabolic profile changed in *A. sapota* fruits. Further studies are required to be carried out to know the proper biochemical reason for such changes.

Conclusion

Metabolomic approaches are increasingly been used to thoroughly study the metabolic composition of plant organs and to characterize the variation in metabolite content (Schauer and Fernie, 2006). Metabolomic approaches to discover the metabolites and metabolic associations correlated with food quality traits are also being used for food crops (Hall *et al.*, 2006; Fernie and Schauer, 2009). The present study used a GC-MS based metabolomics approach to demonstrate how the characteristic metabolic profile of sapodilla fruit varied during the postharvest ripening period. The present data revealed, for the first time, not only the detailed composition of *A. sapota* fruit metabolites but also changes of individual metabolites during postharvest ripening. On the whole it appears that in spite of decrease in the level of many metabolites, a large number of amino acids and sugar alcohols increase in quantity. In conclusion, the obtained results from the GC-MS based metabolic data indicated that *Achras sapota* fruit contains a lot of health beneficial metabolites. On the basis of metabolites that could be identified during the study, it is suggested that the fruit quality is ameliorated during postharvest ripening.

Acknowledgements

The authors are grateful to the Agricultural Field Station of University of Calcutta, Baruipur, India for supplying the cricket ball variety of *Achras sapota* fruits. SD acknowledges financial assistance from University Grant Commission, India (Faculty Development Programme – Teacher Fellowship Award). Financial support from DST (FIST) is also acknowledged.

References

Carrari, F. and Fernie A.R. 2006. Metabolic regulation underlying tomato fruit development. Journal of Experimental Botany 57: 1883-1877.

Das, S., Das, S. and De, B. 2012. In vitro inhibition of key enzymes related to diabetes by the aqueous extracts of some fruits of West Bengal, India. Current Nutrition and Food Science 8(1): 19-24.

Das, S. and De, B. 2013. Evaluation of Angiotensin I-Converting Enzyme (ACE) inhibitory potential of some underutilized indigenous fruits of West Bengal using an in vitro model. Fruits 68(6): 499-506.

Debolt, S., Cook, D.R. and Ford, C.M.L. 2006. Tartaric acid synthesis from vitamin C in higher plants. Proceedinings of the National Academy of Sciences of the United States of America 103: 5608-5613.

Deluc, L.G. Grimplet, J., Wheatley, M.D., Tillett, R., Quilici, D.R., Osborne, C., Schooley, D.A., Schlauch, K.A., Cushman, J.C. and Cramer G.R. 2007. Transcriptomic and metabolite analyses of Cabernet Sauvignon grape berry development. BMC Genomics 8: 429.

Fernie, A.R. and Schauer, N. 2009. Metabolomics – assisted breeding: a viable option for crop improvement? Trends in Genetics 25(1): 39-48.

Giovannoni, J.J. 2004. Genetic regulation of fruit development and ripening. Plant Cell 16 (Suppl): S170–S180.

Giovannoni, J.J. 2007. Fruit ripening mutants yield insights into ripening control. Current Opinion in

Plant Biology 10: 283–289.
Hall, R.D., Brouwer, I. and Fitzgerald, M.A. 2008. Plant metabolomics and its potential for human nutrition. Physiologia Plantarum 132(2): 162-175.
Haynes, T.E., Li, P., Li, X.L., Shimotori, K., Sato, H., Flynn, N.E., Wang, J.J., Knabe, D.A. and Wu, G. 2009. L-glutamine or L-alaninyl-L-glutamine prevents oxidant- or endotoxin- induced death of neonatal enterocytes. Amino Acids 37: 131-142.
Kim, H., Park, S.J., Hyun, S., Yang, S., Lee, J., Auh, J., Kim, J., Cho, S., Marriott, P.J. and Choi, H. 2011. Biochemical monitoring of black raspberry (*Rubus coreanus* Miquel) fruits according to maturation stage by 1H NMR using multiple solvents. Food Research International 44: 1977-1987.
Lee, S., Choi, H., Cho, S.K. and Kim, Y. 2010. Metabolic analysis of guava (*Psidium guajava* L.) fruits at different ripening stages using different data-processing approaches. Journal of Chromatography B 878: 2983-2988.
Livesey, G. 2003. 2003. Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. Nutrition Research Review 16: 163-191.
Lombardo, V.A., Osorio, S., Borsani, J., Lauxmann, M.A., Bustamante, C.A., Budde, C.O., Andreo, C.S., Lara, M.V., Fernie, A.R. and Drincovich, M.F. 2011. Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. Plant Physiology 157: 1696-1710.
Moco, S.I.A.., Bino, R.J., Vos de, C.H. and Vervoort, J.J.M. 2007. Metabolomics technologies and metabolite identification. Trends in Analytical Chemistry 26(9): 855-866.
Oms-Oliu, G., Hertog, M.L.A.T.M., Van de Poel, J., Ampofo-Asiama Geeraerd, A.H. and Nicolaï, B.M. 2011. Metabolic characterization of tomato fruit during preharvest development, ripening, and postharvest shelf-life. Postharvest Biology and Technology 62(1): 7-16.
Roessner, U., Patterson, J.H., Forbes, M.G., Fincher, G.B., Langridge, F.P. and Bacic, 2006. A. An investigation of Boron Toxicity in Barley using metabolomics. Plant Physiology 142(3): 1087- 1101.
Roessner, U., Wagner, C/, Kopka, J, Trethewey, R.N. and Willmitzer, L. 2000. Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. Plant Journal 23(1): 131-142.
Schauer, N. and Fernie, A.R. 2006. Plant metabolomics: towards biological function and mechanism. Trends in Plant Science 11(10): 508–516.
Shui, G., Wong, S.P., Leong, L.P. 2004. Characterization of antioxidants and change of antioxidant levels during storage of *Manilkara Zapota* L. Journal of Agricultural and Food Chemistry 52(26): 7834-7841.
Stipanuk, M.H., Dominy, J.E., Lee, J.I. and Coloso, R.M. 2006. Mammalian cysteine metabolism: new insights into regulation of cystein metabolism. Journal of Nutrition 136(6 Suppl): S1652-1659.
Sumner, L.W., Mendes, P. and Dixon, R.A. 2003. Plant metabolomics: large scale phytochemistry in the functional genomics era. Phytochemistry 62(6): 817-836.
Wu, G. 2010. Functional amino acids in growth, reproduction, and health. Advances in Nutrition 1: 31-37.
Zhang, J., Wang, X., Yu, O., Tang, J., Gu, X., Wan, X. and Fang, C. 2011. Metabolic profiling of strawberry (*Fragaria x ananassa* Duch.) during fruit development and maturation. Journal of Experimental Botany 62(3): 1103-1118.