Journal of Chromatography B, 878 (2010) 2983–2988

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Metabolic analysis of guava (*Psidium guajava* L.) fruits at different ripening stages using different data-processing approaches

Sarah Lee [a], Hyung-Kyoon Choi [b], Somi Kim Cho [c], Young-Suk Kim [a,*]

[a] *Department of Food Science and Technology, Ewha Womans University, Seoul 120-750, Republic of Korea*
[b] *College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea*
[c] *College of Applied Life Sciences, Jeju National University, Jeju 690-756, Republic of Korea*

ARTICLE INFO

Article history:
Received 18 December 2009
Accepted 2 September 2010
Available online 21 September 2010

Keywords:
Guava fruit
Ripening
Flesh/peel
Gas chromatography time-of-flight mass spectrometry (GC-TOF/MS)
Principal component analysis (PCA)
Data processing

ABSTRACT

Gas chromatography coupled with time-of-flight mass spectrometry and principal component analysis were used to obtain the metabolite profiles of guava (*Psidium guajava*) fruits. Results with two types of data-processing software, ChromaTOF and AMDIS, were compared to explain the differences between the samples. There were some differences in score and loading plot patterns of PCA as well as in the composition of the metabolites. However, little difference was observed in the type of metabolites detected and identified using either type of software. Both the flesh and peel of premature and mature white guava fruits were compared for the analysis of the metabolite profiles. Malic acid, aspartic acid, and glucose were the major metabolites distinguishing the different parts of guava fruits in the PCA loading plot. In addition, the metabolic profiles of the fruits revealed significant changes in some metabolites during ripening. The major components contributing to the separation were serine, citric acid, fructose, sucrose, and some unknowns. In particular, sucrose, fructose, serine and citric acid were related to the ripening of guava fruits. Fructose and sucrose were increased whereas citric acid was decreased during guava fruit ripening.

1. Introduction

Guava, *Psidium guajava* L. (*myrtaceae*), is a tropical fruit tree that is mainly grown in South America, North Africa, and Southeast Asia [1,2]. Guava fruit is round, ranging from 3 to 10 cm in diameter, and has a yellow or pink peel at maturity in some species [3]. It is widely consumed fresh but also within processed products (e.g., juice, tea, ice cream, jam) because of its palatable flavor and taste as well as containing various nutritional benefits for the consumer [4]. Previous studies on guava fruits have demonstrated that physical and chemical changes, such as appearance, hardness, and chemical compositions of phenolic compounds, sugars, and ascorbic acid, occur during maturation [5–7]. Knowledge about the chemical changes of fruits is important for a better understanding of metabolic processes such as ripening. Total soluble solids, total sugar content, and ascorbic acid have been shown to significantly increase as fruit ripens [5].

Although some studies have addressed the metabolic changes of guava fruits during ripening, they were focused only on targeted characteristics or compounds such as acidity, sugars, or ascorbic acid [5,8]. No study has addressed the diverse range of metabolites of guava fruits during maturation using metabolomic approaches.

In metabolomic studies, gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF/MS) approach has been widely used for analyzing metabolites from a range of biological samples, including plant extracts [9–15]. Considering the advantages of this technique, such as relatively high reproducibility, high resolution, high-throughput analysis and good sensitivity, GC-TOF/MS has mainly been used to identify differences in metabolite profiles between samples [16–19]. In addition, TOF/MS has other advantages such as its fast analyte detection and deconvolution process [20]. Deconvolution of peaks obtained in the data sets of complex samples is an important part of metabolic analysis using TOF/MS. Freely available (e.g., AMDIS, XCMS, and MZmine) or commercial (e.g., MarkerLynx, ChromaTOF, and Lineup) software can provide deconvolution functions such as peak selection and peak alignment for peak identification and quantification [21]. These software programs can minimize the time spent on analysis and allow more time for identifying metabolites, that are related to differences between samples. Although this method has many advantages as explained above, it is required to compare the results obtained with GC-TOF/MS data sets using different software programs with different platforms. The data pre-treatment approaches described and used within the current study are also necessary to help understand the metabolic

* Corresponding author. Tel.: +82 2 3277 3091; fax: +82 2 3277 4213.
E-mail address: yskim10@ewha.ac.kr (Y.-S. Kim).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.09.003

changes that occur within complex biological (guava fruit) samples.

To date, comparisons of data-processing software have involved only standard solutions or targeted compounds. No study has focused on the comparison of different software programs analyzing biological samples such as plants. The objective of this study was to compare the results obtained using different data-processing procedures with two software programs, ChromaTOF and AMDIS (Automated Mass Spectral Deconvolution and Identification System). The present study was based on GC-TOF/MS acquired data sets generated to help understand the levels and variations of key metabolites in different parts of guava fruits during ripening.

2. Experimental

2.1. Materials

White guava fruits (*P. guajava*) cultivated in Cheju Island, South Korea were harvested at premature and mature stages in September and October 2007, respectively. Premature fruits had a green peel and hard texture. Mature fruits were obtained at the final harvest period, had a pale yellow peel, soft texture, and a highly sweet flavor, and were bigger (2.5–3 cm in diameter) than premature fruits (1.5–1.8 cm in diameter). All of the guava fruits were cleaned with distilled water and wiped with paper. Three fruits from each ripening stage were selected based on similar size and peel color. Guava fruits were separated into peel (exocarp) and flesh (mesocarp). The whole fruit was cut into two portions. Then, the flesh was taken out using a spoon whereas the peel was obtained after the remaining flesh part was further removed. Many seeds were embedded in the flesh because they could not be separated from the flesh in the fruit. After the peel was separated from the flesh, both parts were immediately freeze-dried and stored at below −70 °C. Each sample was homogenized with a mortar and pestle before extraction.

2.2. Sample extraction and derivatization

The ground powder (100 mg) was extracted with 10 mL of 80% methanol containing 2 μL of an internal standard compound (ribitol, 0.02 g/mL in 80% methanol). The extraction was performed using an ultrasonicator (Branson, Danbury, CT, USA) for 30 min. After extraction, the extract was centrifuged at 3000 rpm for 10 min. Then, 200 μL of the supernatant was completely dried in a Centri-Vap (Labconco Co., Kansas City, MO, USA) for 10 h. For oximation and derivatization, 10 μL of methoxyamine hydrochloride (20 mg/mL) in pyridine was added to the dried extract and oximated at 30 °C for 90 min. After that, the oximated samples were silylated with 40 μL of N-methyl-N-trifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS) (Pierce, Rockford, IL, USA) at 37 °C for 30 min.

2.3. GC-TOF/MS analysis

An Agilent 6890N GC system (Palo Alto, CA) equipped with an Agilent 7683 autosampler was coupled to a time-of-flight Pegasus III mass spectrometer (Leco, St. Joseph, MI, USA), operating in electron ionization (EI) mode (70 eV). A DB-5MS column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA) was used with helium at a constant flow of 1.0 mL/min. 1 μL of the derivatized sample was injected with a split ratio 100:1. The oven temperature was programmed as follows: 80 °C (2 min); 15 °C/min to 180 °C; 4 °C/min to 250 °C (2 min); 15 °C/min to 300 °C (5 min). The acquisition rate was set to 20 scans s^{-1} with a mass scan range of m/z = 45–550. The injector and transfer line temperatures were 230 °C and 250 °C, respectively.

2.4. Mass spectral data processing

Two different data processing methods using the ChromaTOF and AMDIS software were employed. The ChromaTOF software was installed in the LECO Pegasus TOF/MS instrument system, whereas AMDIS was freely available software that was obtained from the website (http://chemdata.nist.gov/mass-spc/amdis/).

Data processing and identification of metabolites were carried out using ChromaTOF software (Leco, 2007). This automatically detected all peaks and deconvoluted the retention times and mass spectra, for identification using an on-line library such as Wiley mass spectral database (Hewlett-Packard Co., 1995), NIST05 MS Library and MS Search Program V.2.0d (NIST, 2005) and an in-house library, in each MS data sets of single run, whereas peak selection and alignment of deconvoluted metabolites were made manually combining the different sets of MS data. Deconvolution parameters were set to the Leco default values. Peaks obtained with a signal-to-noise ratio (S/N) lower than 10 were rejected. The baseline offset was set to 1.0. The data point for averaging was set to auto, and the peak width was set to 1.333. The identification of each metabolite was confirmed by comparison of retention times and mass spectra with those of authentic chemicals, and semi-quantification was made by comparison of peak areas of each metabolite with that of the internal standard. The other data processing approach used was the AMDIS (Automated Mass Spectral Deconvolution and Identification System) software. This software has been used in automated-mass-spectral deconvolution and baseline correction. Non-processed data files were converted to the ANDI MS*.cdf file format and uploaded to the AMDIS software. The deconvolution parameters were chosen to obtain the maximum number of metabolites, excluding peak noises. In general, as a number of metabolites deconvoluted were increased, sample groups were more clearly separated in PCA score plot. Therefore, the settings leading to a maximum of number of detected metabolites were considered to be optimum. Three parameters, resolution, sensitivity and peak shape, were compared and operated from low to high, respectively (data not shown). As resolution and sensitivity parameters increased from low to high, the number of deconvoluted components increased. On the other hand, as the shape requirements were changed from low to high, the number of deconvoluted components decreased. When parameters were set to a lower number of components for deconvolution, the number of correctly deconvoluted spectra also increased. Although AMDIS software could sometimes report a few false positives to identify more peaks, the accuracy of metabolites could be increased by adjusting the parameters and combining with Spectconnect programs. The component width set to 12, adjacent peaks subtraction set to 1, resolution set to high, sensitivity set to low, and shape requirements set to high. The MS data results were processed with AMDIS software and ELU files were created as outputs. The Spectconnect program (http://spectconnect.mit.edu) was also employed to align batches of ELU files from related chromatograms and to filter peaks.

2.5. Statistics

Multivariate data analysis was performed using the SIMCA-P+ 11.0 software (Umetrics AB, Umea, Sweden). Principal component analysis (PCA), an unsupervised clustering method, was performed and data were centered and scaled using unit variance (UV) scaling. PCA was used to compare data processing results in guava samples of different parts at premature and mature stages.

3. Results and discussion

3.1. Comparison of data processing

The GC-TOF/MS instrument has been widely applied in metabolomic studies due to its higher mass accuracy and mass resolution relative to quadrupoles [22]. In addition, the high sensitivity and low detection limit of GC-TOF/MS allows for the detection of a wide variety of metabolites. However, tremendous amounts of data that result from GC-TOF/MS analysis are difficult to resolve completely. Therefore, deconvolution is needed to obtain accurate mass spectra and resolve the overlapping chromatographic peaks [23]. Lu et al. examined a deconvolution technique with test-mixture solutions containing authentic standards using three data-processing software programs, ChromaTOF, AMDIS, and AnalyerPro. According to their results, both AMDIS and ChromaTOF software were the most efficient and flexible data-handling programs tested. for obtaining more accurate mass spectra results [24].

As Fig. 1 illustrates, there were some differences in the final PCA results between the ChromaTOF and AMDIS data-processing softwares.

After data processing, 42 and 33 significant components, contributing to separate guava fruits according to different ripening stages and parts, were deconvoluted using ChromaTOF and AMDIS, respectively. This is clearly due to the different applied deconvolution and alignment parameters. Data processing software have their own parameters to optimize the results. AMIDS produced a clearer separation of the samples but yielded lower total variability (47.9%) in the principal component analysis (PCA) score plot. Although similar types of metabolites were detected using both software programs, AMDIS coupled with the SpectConnect program, which has an automated processing system, was more convenient and faster in finding and identifying diverse metabolites.

3.2. Classification of guava fruits by PCA

After data processing, the GC-TOF/MS data sets were subjected to multivariate data analysis using PCA. PCA is an unsupervised method that reduces a large data matrix to a few composite variables, called principal components, for visualization and interpretation of data [25]. It was difficult to deal with many samples using manual software, ChromaTOF, which required time-consuming work to merge the complex data sets. Accordingly, only three fruits from each ripening stage could be selected based on similar size and peel color to obtain GC-TOF/MS data sets.

As Fig. 1 shows, guava fruits were separated by PCA according to not only different parts (flesh and peel) but also different ripening stages. The first principal component (PC1) mainly explained the ripening stages whereas the second principal component (PC2) was related to the different parts of guava fruits (PC2).

With ChromaTOF software data processing (Fig. 1a), approximately, more than 150 extracted MS peaks were obtained by using ChromaTOF software. Among them, 42 variables were considered as significant in the separation of guava fruits according to different ripening stages and parts. The PC1 and 2 scores could explain 53.6% of the total variability. Mature fruits (positive PC1 dimension) could be separated from premature fruits (negative PC1 dimension) mainly in the score of PC1, while fruit flesh (negative PC2 dimension) were separated from fruit peel (positive PC2 dimension) in PC2. The major metabolites contributing to the PC1 dimension were fructose, sucrose, serine and citric acid. In contrast, the important metabolites of the PC2 dimension were malic acid and aspartic acid. The results of the AMDIS data processing were similar to those of ChromaTOF software data processing. The PC1 score, which explained 26.3% of the total variability, allowed for the separation of ripening stages, whereas the PC2 score, which explained 21.6% of the total variance, could separate the flesh and peel of guava fruits (Fig. 1b).

Citric acid, fructose, sucrose and some unknowns were found to be the variables responsible for the major source of variation (PC1) within the model. Malic acid and aspartic acid were two of the components that contributed to the variation observed along PC2.

3.3. Metabolic characterization of guava fruits according to different periods and parts

Table 1 shows the major metabolites that contributed to the different ripening stages and parts of guava fruits. Glucose and fructose have a carbonyl group (C=O) and 5-6 hydroxyl groups (–OH) in their chemical structures. For derivatization, the carbonyl group is protected by oximation, forming two methoxime isomers (E/Z or syn/anti) [26]. Then, polar compounds containing functional groups, such as –OH, –SH, or –NH groups, were added a trimethylsilyl (TMS) group for derivatization, Accordingly, the detection of glucose 1–4 and fructose 1 and 2 could be the result of the two methoxime isomers or different positions of trimethylsilyl groups. Multiple derivatized forms of both sugars were combined together to obtain the quantification results in this study.

The levels of fructose, sucrose and alanine in mature fruit flesh were 1.5–2 times higher than those in premature fruit flesh whereas citric acid and aspartic acid were more than 2 times higher in premature fruit peel compared to mature fruit peel. As expected, the levels of both glucose and fructose increased significantly in the mature flesh of guava fruits. Some studies have shown that the level of fructose and other reducing sugars increases during guava fruit ripening [5,27].

One of the most significant changes associated with ripening of fruits is reduced acid content. Malic acid and citric acid are the predominant organic acids related to fruit ripening [28]. In particular, citric acid is the major compound that contributes to the acidity of fruit [29]. In guava fruits in the current study, both citric acid and malic acid were dominant among diverse organic acids. However, the level of citric acid decreased with fruit ripening while malic acid content exhibited the opposite trend.

In mature fruits, sucrose, glucose, γ-aminobutyric acid (GABA), serine, and alanine were more abundant in flesh than in peel. In contrast, malic acid and citric acid were higher in peel than in flesh. The levels of fructose, sucrose and alanine were 1.5–2 times higher both flesh and peel in mature fruits. In both premature and mature fruits, the peel had higher levels of citric acid and malic acid than the flesh whereas aspartic acid and glucose were more abundant in flesh. In particular, premature peel of guava fruits contained much higher levels of citric acid. In contrast, aspartic acid was the main contributors to premature flesh in guava fruits.

3.4. Ripening mechanism of guava fruit

Fruits exhibit relatively high metabolic activity related to development and ripening such as synthesis of novel proteins, new pigments and flavor compounds. During ripening, fruits changes in color, texture and flavor, indicating that compositional changes are taking place. These activities require both energy and a supply of carbon-skeleton building blocks. Respiration is one of the major factors in fruit ripening, and two major respiratory substrates are sugars and organic acids [30]. In fruits, the most common sugars are fructose, glucose, and sucrose whereas the most prevalent organic acids are citric acid and malic acid. Based on the results of this study, sugars such as fructose, glucose, and sucrose, and organic acids, citric acid and malic acid, were major metabolites contributing to ripening in guava fruit.

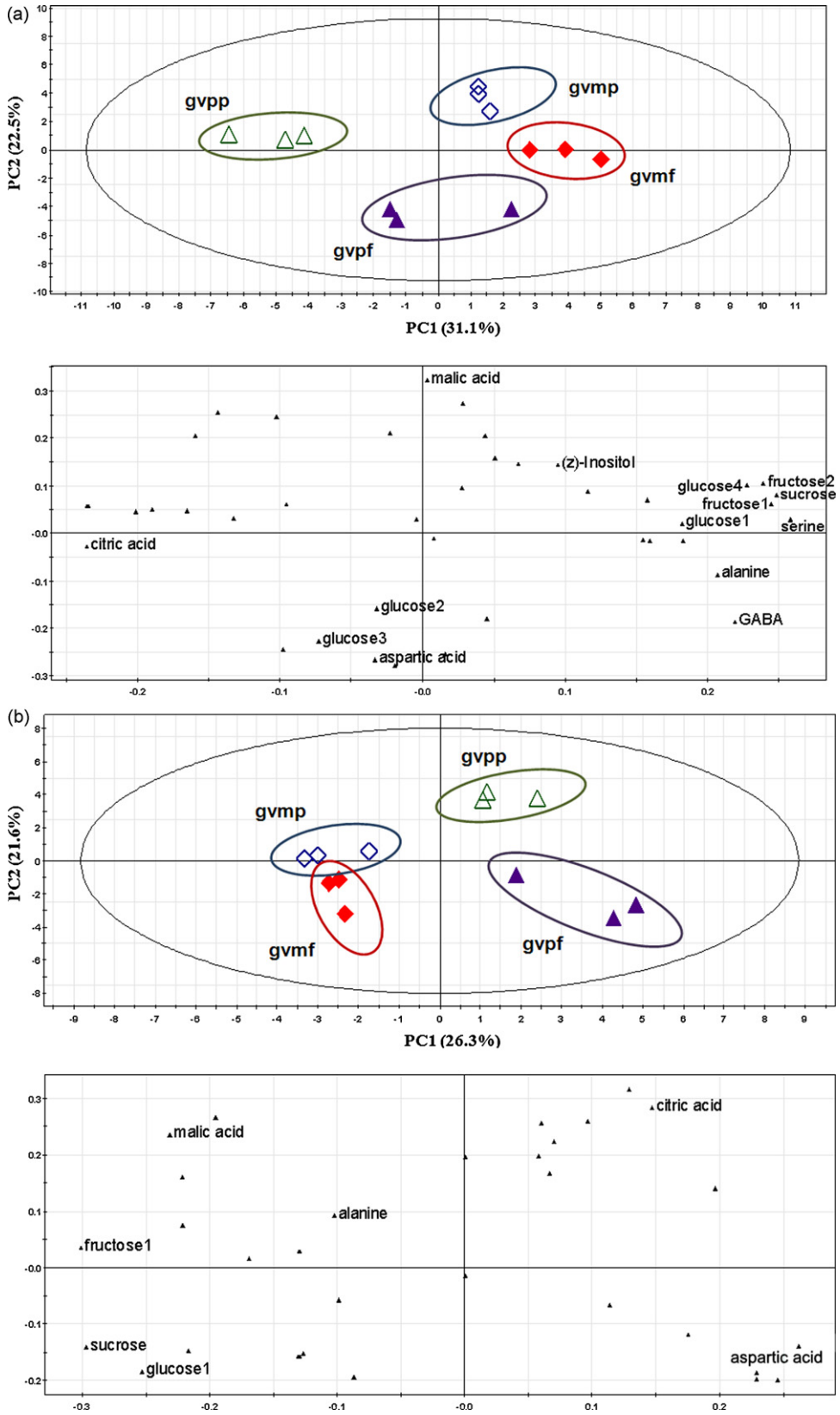

Fig. 1. PCA score and loading plot (a) ChromaTOF data processing and (b) AMDIS data processing in different parts and ripening stages of guava fruit. gvmf: guava mature flesh; gvmp: guava mature peel; gvpf: guava premature flesh; gvpp: guava premature peel.

Table 1
Major metabolites contributing to differences in metabolite profiles of flesh and peel parts at different ripening stages.

Metabolites	Relative peak areas (mean ± SD)[a]			
	gvmf[b]	gvmp[c]	gvpf[d]	gvpp[e]
Alanine	0.0261 ± 0.0031	0.0093 ± 0.0081	0.0134 ± 0.0043	0.0060 ± 0.0016
Serine	0.0035 ± 0.0002	0.0032 ± 0.0002	0.0025 ± 0.0004	0.0012 ± 0.0010
Malic acid	0.0309 ± 0.0026	0.0582 ± 0.0040	0.0103 ± 0.0023	0.0385 ± 0.0022
Aspartic acid	0.0071 ± 0.0006	0.0072 ± 0.0007	0.0639 ± 0.0174	0.0178 ± 0.0021
GABA	0.0079 ± 0.0004	0.0040 ± 0.0002	0.0070 ± 0.0015	0.0025 ± 0.0006
Citric acid	0.9432 ± 0.0418	1.5286 ± 0.0608	2.1673 ± 0.4711	3.3446 ± 0.2277
Fructose	11.3764 ± 0.7541	11.4256 ± 0.7926	9.4415 ± 2.4402	7.8373 ± 0.5896
Sucrose	11.5201 ± 1.1127	8.3776 ± 0.4838	4.4520 ± 3.5025	2.6564 ± 0.2185
Glucose	5.1214 ± 0.4683	4.8102 ± 0.4600	5.6616 ± 1.5903	4.8616 ± 0.4570

[a] Average of relative peak areas to that of internal standard [2 μL of ribitol (0.02 g/mL) in 80% methanol] ($n = 3$) ± standard deviation.
[b] gvmf: guava mature flesh.
[c] gvmp: guava mature peel.
[d] gvpf: guava premature flesh.
[e] gvpp: guava premature peel.

The respiratory pathways used by fruits for the oxidation of sugars occurred via glycolysis, the oxidative pentose phosphate pathway, and the tricarboxylic acid (TCA) pathway. The increased respiration of sugars in fruit seems to be mediated largely by an increased flux through glycolysis [31]. Fruits continue to accumulate sugars during ripening, which account for a large part of the flavor such as tastants. Stored starch in fruit is converted to sugars, leading to the sweet taste of ripened fruits [27]. In guava fruits, stored starch was degraded to sucrose, fructose and glucose and it affected sweetness of fruits. Fructose and sucrose, relatively high in sweetness, were mainly increased in mature fruits. On the other hand, levels of acid are known to decline during ripening, presumably because they are used as a respiratory substrate and generation of ATP [32]. Citric acid and malic acid were the prevalent metabolites in guava fruit ripening in this study. In the TCA cycle, citrate is sequentially metabolized to isocitrate, 2-oxoglutarate and glutamate. Glutamate is catabolized through GABA [33]. Therefore, as would be expected, citrate in the current study was decreased while GABA was increased in guava fruit during ripening. As Fig. 2 shows, sugars such as sucrose, fructose, and glucose and amino acids including alanine and serine increased as a result of glycolysis, while aspartic acid decreased via the TCA cycle, being converted to oxaloacetate and fumarate in guava fruits.

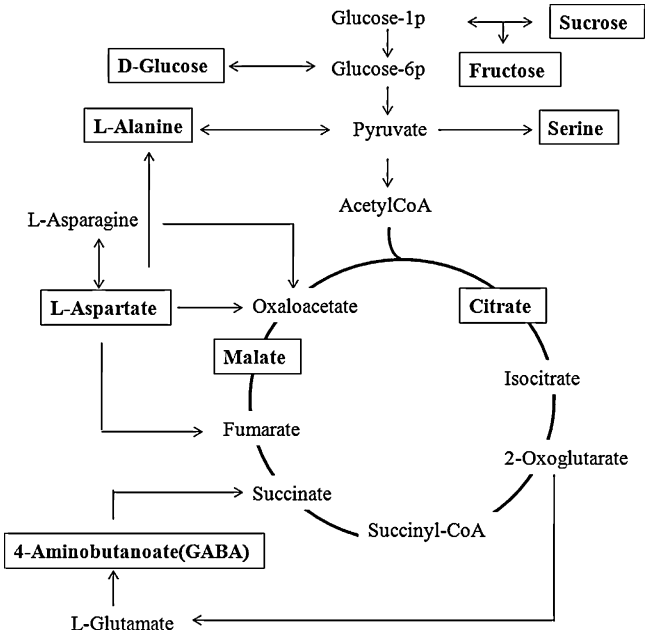

Fig. 2. Schematic of metabolite pathways of guava fruit detected by PCA based on GC-TOF/MS according to different parts and ripening stages. Boxed terms represent major metabolites in guava fruit contributing to different parts and ripening stages. This was modified from pathways presented in Ref. [34].

4. Conclusion

Commercially (ChromaTOF) or freely available (AMDIS) software programs were used for the analysis of metabolites data collected by GC-TOF/MS. The programs demonstrated some differences in score and loading plot patterns of PCA as well as in the composition of the metabolites. However, little difference was observed in the type of metabolites detected and identified using either type of software. Not only the different parts (flesh and peel) but also the different ripening stages of the guava fruits could be clearly separated by PCA, which led to the determination of major metabolites related to different parts of guava fruits at premature and mature stages. To our knowledge, this is the first study that has compared processing software in the field of non-targeted metabolite profiling of plants.

Acknowledgments

This work was supported by a Korea Science and Engineering Foundation (KOSEF) grant funded by the Korean government (MEST) (No. R01-2007-000-20492-0).

References

[1] A. Jimenez-Escrig, M. Rincon, R. Pulido, F. Saura-Calixto, J. Agric. Food Chem. 49 (2001) 5489.
[2] J. Kamath, N. Rahul, C.A. Kumar, S.M. Lakshmi, Int. J. Green Pharm. 2 (2008) 9.
[3] H.T. Chan, in: S. Nagy, C.S. Chen, P.E. Shaw (Eds.), Fruit Juice Processing Technology, Agscience Inc., Auburndale, 1993, p. 334.
[4] R.N. Adsule, S.S. Kadam, in: D.K. Salunke, S.S. Kadam (Eds.), In Handbook of Fruit Science and Technology. Production, Composition, Storage, and Processing, CRC, New York, 1995, p. 419.
[5] R.E. El buluk, F.E. Babiker, A.H. El tinay, Plant Food Hum. Nutr. 49 (1996) 147.
[6] F.D. Soares, T. Pereira, M.c.O. Maio Marques, A.R. Monteiro, Food Chem. 100 (2007) 15.
[7] S. Yusof, S. Mohamed, J. Sci. Food Agric. 38 (1987) 31.
[8] H.A. Bashir, A.-B.A. Abu-Goukh, Food Chem. 80 (2003) 557.
[9] G.S. Catchpole, M. Beckmann, D.P. Enot, M. Mondhe, B. Zywicki, J. Taylor, N. Hardy, A. Smith, R.D. King, D.B. Kell, O. Fiehn, J. Draper, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 14458.
[10] T. Kind, V. Tolstikov, O. Fiehn, R.H. Weiss, Anal. Biochem. 363 (2007) 185.
[11] M. Kusano, A. Fukushima, M. Kobayashi, N. Hayashi, P. Jonsson, T. Moritz, K. Ebana, K. Saito, J. Chromatogr. B 855 (2007) 71.
[12] W. Pongsuwan, E. Fukusaki, T. Bamba, T. Yonetani, T. Yamahara, A. Kobayashi, J. Agric. Food Chem. 55 (2007) 231.
[13] T. Shepherd, G. Dobson, S.R. Verrall, S. Conner, D.W. Griffiths, J.W. McNicol, H.V. Davies, D. Stewart, Metabolomics (2007) 1573.
[14] S. Tianniam, L. Tarachiwin, T. Bamba, A. Kobayashi, E. Fukusaki, J. Biosci. Bioeng. 105 (2008) 655.

[15] Y. Tikunov, A. Lommen, C.H.R. de Vos, H.A. Verhoeven, R.J. Bino, R.D. Hall, A.G. Bovy, Plant Physiol. 139 (2005) 1125.
[16] S. Abate, Y. Ahn, T. Kind, T. Cataldi, O. Fiehn, Rapid. Commun. Mass Spectrom. 24 (2010) 1172.
[17] C. Denkert, J. Budczies, T. Kind, W. Weichert, P. Tablack, J. Sehouli, S. Niesporek, D. Koensgen, M. Dietel, O. Fiehn, Cancer Res. 66 (2006) 10795.
[18] K.M. Pierce, J.L. Hope, J.C. Hoggard, R.E. Synovec, Talanta 70 (2006) 797.
[19] C. Wagnera, M. Sefkowb, J. Kopkaa, Phytochemistry 62 (2003) 887.
[20] Z.D. Peterson, C.R. Bowerbank, D.C. Collins, S.W. Graves, M.L. Lee, J. Chromatogr. A 992 (2003) 169.
[21] M. Katajamaa, M. Oresic, J. Chromatogr. A 1158 (2007) 318.
[22] M. Bedair, L.W. Sumner, Trends Anal. Chem. 27 (2008) 238.
[23] P. Jonsson, J. Gullberg, A. Nordstrom, M. Kusano, M. Kowalczyk, M. Sjostrom, T. Moritz, Anal. Chem. 76 (2004) 1738.
[24] H. Lu, W.B. Dunn, H. Shen, D.B. Kell, Y. Liang, Trends Anal. Chem. 27 (2008) 215.
[25] J. Xing, W. Saeys, J. De Baerdemaeker, Comput. Electron. Agric. 56 (2007) 1.
[26] R.A. Laine, C.C. Sweeley, Anal. Biochem. 43 (1971) 533.
[27] V.V.R. Subramanyam, K.T. Acharya, J. Sci. Food Agric. 8 (1957) 657.
[28] H.-D. Belitz, W. Grosch, Food Chemistry, Springer-Verlag, Heidelberg, 1987, p. 592.
[29] W.B. Sinclair, R.C. Ramsey, Bot. Gaz. 106 (1944) 140.
[30] G.A. Tucker, D. Grierson, in: D.D. Davies (Ed.), Fruit Ripening, Academic Press, 1987, p. 265.
[31] G.B. Seymour, J.E. Taylor, G.A. Tucker, in: G.A. Tucker (Ed.), Biochemistry of Fruit Ripening, Chapman & Hall, London, 1993, p. 1.
[32] R. Ulrich, in: A. Hulme (Ed.), The Biochemistry of Fruits and their Products, Academic Press, 1970, p. 89.
[33] F. Gallardo, S. Gálvez, P. Gadal, F.M. Cánovas, Planta 196 (1995) 148.
[34] KEGG Database, Kanehisa Laboratories, Tokyo, 2009.