Vol. 16(6), pp. 111-124, July 2022
DOI: 10.5897/AJPS2022.2246
Article Number: 02A407C69431
ISSN 1996-0824

http://www.academicjournals.org/AJPS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Plant Science

Full Length Research Paper

Identification and relative quantitative comparison of compounds in bottle gourds [*Lagenaria siceraria* (Mol.) Standl.]

Zhaohui Zhang[1], Xuan Du[2], Na Liu[2] and Chunhong Chen[1]*

[1]Shanghai Academy of Agricultural Sciences, Zhuanghang Comprehensive Experiment Station, Shanghai, 201403, China.
[2]The Protected Horticulture Institute, Shanghai Academy of Agricultural Sciences, Shanghai Key Laboratory of Protected Horticultural Technology, Shanghai, 201403, China.

Received 25 March, 2022; Accepted 30 June, 2022

To study the diversity and cultivar-specific of phytochemicals in bottle gourd, Gas chromatography-mass spectrometry (GC-MS)-based untargeted metabolomics method was used to analyze the metabolic profiles of two groups of bottle gourd cultivars exhibiting different tastes and flavors: fresh and slightly bitter (BBGs) / fragrant and sweet (SBG). A total of 155 metabolites (16 differential metabolites and 139 non-differential metabolites) were identified/annotated. The relative contents of 16 differential metabolites in BBGs are all higher than that in SBGs. The differential metabolites profiles of the two different groups were distinguished using principal component analysis (PCA), and the main differential metabolic pathways between the BBG and the SBG included those relating to plant nutrition and energy metabolism. This study provides new insights into the differences in metabolite profiles among bottle gourds with different tastes and flavors.

Key words: Gas chromatography-mass spectrometry (GC-MS), metabolomics, bottle gourd, taste, flavor.

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] (2n = 2x = 22), also known as long calabash, is an annual cucurbit crop, and its tender fruit is cooked as a vegetable because of its unique flavor (Wu et al., 2017). The bottle gourd contains essential nutrients, such as protein, vitamin, pectin, fibers, etc (Zhang et al., 2020). It is planted in Asia and Africa, as well as it is used in crafts, decorations, and medicinal (Xu et al., 2011). It can be used as the rootstock of other Cucurbitaceae, such as watermelon (Sari, 2003).

The fruits of different gourd varieties have different shapes, sizes and flavors (Wu et al., 2017; Zhang et al., 2020). These phenotypic differences are caused by gene transcription regulation (Zhang et al., 2020), and differences at gene level led to differences at metabolic level. There are few studies on the differences of metabolism of different gourd fruits.

Sugars, amino acids (Kader et al., 1978), organic acids

*Corresponding author. E-mail: Chenchunhong2021@126.com, yyliuna@163.com.

Zhang Zhaohui and Du Xuan contributed equally to this article.

(Robert and Franco, 2018), vitamins (Kafkas et al., 2006) components of plant organisms, but also the ratio of their contents to their contents affects the taste and flavor of edible parts of plants, such as fruits (Hobson and Bedford, 2015). The tastes that human beings can perceive are sweet, sour, bitter, salty and delicious. Generally speaking, the taste of the fruit is caused by its organic components. And traditionally, sugar content determines the sweetness of the fruit. However, with the continuous discovery of new organic compounds and technological progress, sugar is not the only substance with a sweet taste. For example, thaumatin is a kind of protein with a sweeter taste than sucrose (Healey et al., 2017). In addition, the content of organic acids and their derivatives in fruit pulp has important dietary considerations, which affect the taste of fruit and the applicability of processing into various fruit products in some cases. It can be seen that whether the fruit is sweet or sour has a great relationship with the proportion of sweet and sour substances in the fruit. The taste of tomato was the first concern and a balanced sugar-acid ratio was preferred in cherry tomatoes (Hobson and Bedford, 2015). Cucurbita pepo contains the toxic tetracyclic triterpenoid compound cucurbitacin, which is the cause of bitterness and toxicity (Verma and Jaiswal, 2015). The factors affecting fruit umami are free amino acids, nucleotides (monophosphates of inosinate or guanylate, inosine 5′-monophosphate, and guanosine-5′monophosphate), short peptides and organic acids (Kurihara, 2009; Wu et al., 2017). Previous studies have found that, free glutamate (Glu) was found to have the most significant association with umami taste, suggesting that free Glu was the main umami-conferring ingredient of bottle gourd (Wu et al., 2017).

Although the above-mentioned research shows that free glucose is an important factor affecting the fresh taste of bottle gourd fruit, there are many factors involved in the taste of the fruit, and the metabolomics analysis of bottle gourd with different tastes can reveal the metabolic reasons for this difference. However, there is no report on the metabolism of bottle gourd fruit taste. Therefore, in order to find out the difference of metabolism between two kinds of bottle gourd fruits with different tastes and provide a theoretical basis for quality breeding of bottle gourd, we collected and analyzed the metabonomic information of slightly bitter bottle gourd (BBG) and sweet bottle gourd (SBG).

MATERIALS AND METHODS

Plant materials and chemicals

Five bottle gourd cultivars were cultivated in a randomized field plot according to standard agricultural practices in a field at the Zhuanghang comprehensive experimental station (E 121°28, N 30°57′) of the Shanghai Academy of Agriculture Sciences in 2019. Two bitter-tasted bottle gourd cv. BG27 and BG32, and three sweet-tasted bottle gourd cv. BG33, BG56, and BG58 were cultivated. They were sowed in March and planted in April. The photon flux density was range 650~850 $W.m^{-2}$. The temperature and relative humidity of the cultivation environment were about 10~25°C and 50~70%, respectively. The fruits were selected 14 days after simultaneous pollination, having a length of 20-35 cm and a diameter of 4.2-5.8 cm. Three marketable immature fruits were harvested for each cultivar and homogenized with equal weight to form a sample. All samples were stored at −80°C until analysis.

Chromatographic grade methanol and chloroform were purchased from Merck Chemicals (Germany). All of the chemicals, such as pyridine, methoxyamine hydrochloride, and N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), and the reference standards used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-2-Chlorophenylalanine at 3.1 mg ml^{-1} in water was prepared and used as internal standards (IS).

Sample preparation and extraction for metabolomic analysis

An aliquot of 1 g bottle gourd sample and 10 ml methanol-water (7:3 v/v) were mixed for metabolite extraction at 60 Hz for 180 s on a tissuelyzer. Further, the mixtures were ultrasonically extracted for 40 min and stored at 4°C for 24 h. After centrifugation at 12,000 × g for 10 min, 400 µl of the supernatant was spiked with 4 µl of the IS, L-2-chlorophenylalanine, and dried completely in a vacuum concentrator. An aliquot of 80 µl methoxyamine hydrochloride (15 mg/ml in pyridine) was added to the residue and incubated at 37°C for 90 min for methoxyamination. Subsequently, the sample was trimethylsilylated by adding 80 µl BSTFA (with 1% TMCS) and incubated at 70°C for 60 min. The derivatized samples were cooled to room temperature before being analyzed (Jaeger, 2008).

GC/MS conditions

For each cultivar, three biological replicates were independently analyzed. In total, 15 samples were randomly analyzed to reduce analysis bias. Under the adjusted initial pressure, all samples were analyzed in randomized order by a 7890B gas chromatography coupled with a 5977B mass spectrometer (Agilent Inc., CA, USA). A DB-5ms capillary column (30 m ×250 µm inner diameter, 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was used to separate compounds. The injector port was heated to 280°C and injections (1 µl) were performed with a split ratio of 5:1. Helium (purity > 99.999%) was used as the carrier gas at a constant flow of approximately 1 ml/min. The column temperature was held at 60°C for 1 min, then increased to 300°C at 5°C/min, and held for 11 minutes. The total run time was 60 min. The temperatures of the transfer line, ion source and quadruple were maintained at 280, 230 and 150°C, respectively. Electron impact ionization mass spectra were recorded with an ionization energy of 70 eV and EM voltage of 970 V. Mass spectra were scanned from 33 to 600 amu in total ion chromatogram mode after a solvent delay of 6.5 min (Weckwerth et al., 2004).

Data processing and statistical analysis

Metabolite identification was performed by the National Institute of Standards and Technology (NIST) mass spectral library (2017) in MSD ChemStation (version E.02.02.1431; Agilent Inc., CA, USA). The raw data acquired by Agilent GC/MS were imported into ChromaTOF (version 4.50.8.0; Leco Corporation, MI, USA) in NetCDF format. ChromaTOF could automatically compute baseline, find peaks above a signal to noise (S/N) of 100:1, deconvolute (identify overlapped peaks), integrate using specific masses, and align the same compound in different samples. The resulting

Figure 1. Five bottle gourd cultivars.
Source: Authors

three-dimensional dataset comprised sample information, peak retention time, and peak intensity. Some artificial peaks generated by noise, column bleed, and by-products in the silylation procedure were removed manually from the dataset. The resulting data were normalized to the area of the IS (L-2-chlorophenylalanine) for further statistical analysis. L-2-chlorophenylalanine was also utilized to assess process variability during sample preparation and data processing (Weckwerth et al., 2004).

For differential metabolites, we usually screen through VIP, such as screening out variables with VIP greater than 1.5, then calculating p-value, further screening out variables less than 0.05, and then observing these folds change was 2.0. Metabolites have been used for principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) by R (www.r-project.org/) to study metabolite cultivars-specific accumulation according to the described (Wang et al., 2018).

To further illustrate the biological significance associated with bitter/sweet taste, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to link differential metabolites to metabolic pathways in the SBGs compared with those in BBGs. Enrichment P-values were computed from a hypergeometric distribution. A p-value < 0.01 was selected to reduce the false discovery rate. The software used for drawing and data analysis was GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Differences in morphologies among the fruits of the five bottle gourd cultivars

These bottle-gourd cultivars were divided into two groups based on their taste and flavor. One group had two members, BG27 and BG32, which tasted lightly bitter (BBGs). The other had three ones, BG33, BG56, and BG58, which tasted thick sweet (SBGs). Though the two groups of bottle gourd cultivars were planted simultaneously and grown in the same field and under the same conditions, not only the taste of the fruits was distinct, but the shape and peel color also appeared different. The shape of BG27 was like Corbel. BG32 had a round body with a long neck. The shape of BG33 was like a long gun. Both BG56 and BG58 were like Cylinders. The peel color of the five bottle gourd cultivars belonged to a different degree of green (Figure 1).

Metabolic profiling

As is mentioned above, a few differences in tastes and shape between the two groups of bottle gourd cultivars existed. While as we all know the soluble solids content and acid-base titration method, which were referred to rough judgments, sometimes cannot exactly respond to the real ones. To make sure the differences in total contents and also in composition, we applied a newly developed GC–MS-based widely untargeted metabolomics method (German et al., 2005). A total of 155 metabolites (16 differential metabolites and 139 non-differential metabolites) were identified/annotated (Table 1; Supplementary S1). The results of all detected metabolites are shown in an Excel file (Supplementary S1), which indicates the differences in the metabolite levels of the five cultivars. To gain more insight into the metabolic differences between BBG and SBG, differential metabolite screening was performed among all 155 metabolites identified/annotated according to the fold-change and the variables determined to be important in the projection (VIP) scores. A fold-change score ≥2 or ≤0.5 among the metabolites with a VIP value>1 was used as an identification criterion. The screening results have been illustrated using volcano plots (Figure 2A). The overall situation of relative expression of 16 differential metabolites is shown by heat map visualization (Figure 2B).

We detect 6 different metabolites of saccharides,

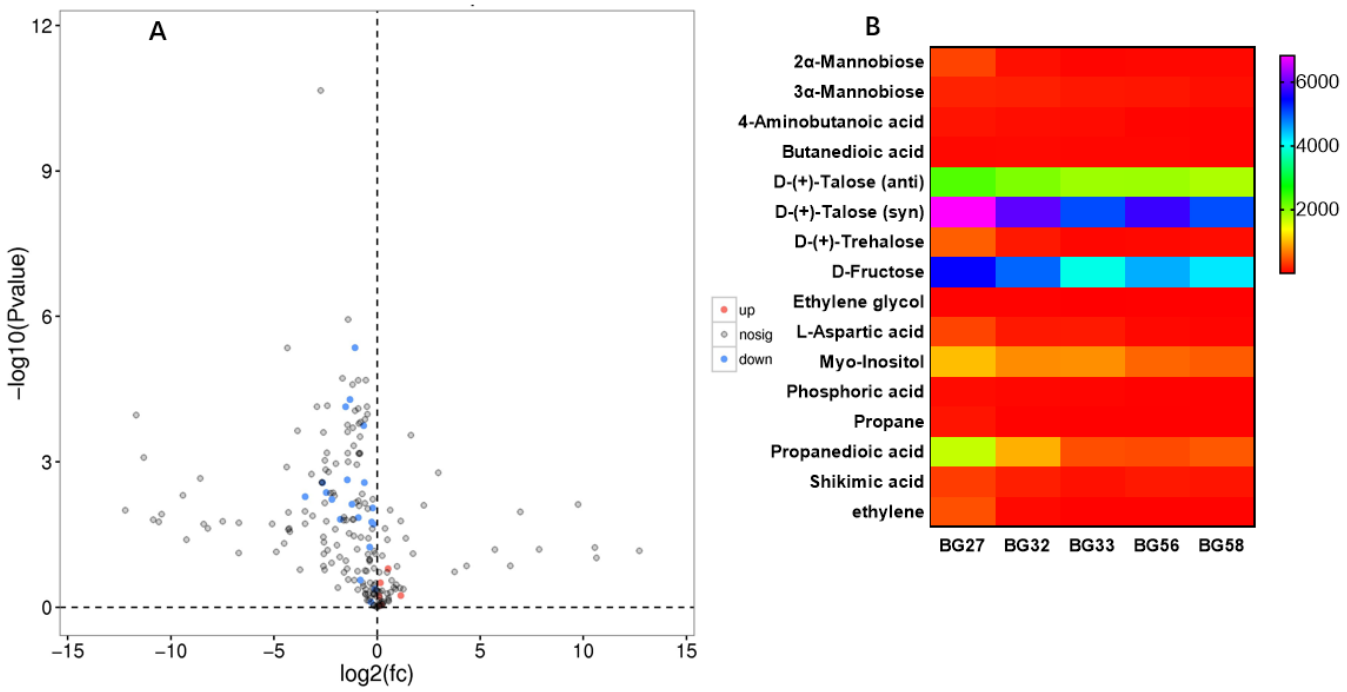

Figure 2. The volcano plot (A) shows the differential metabolite expression levels between BBGs and SBGs. Blue dots represent downregulated differentially expressed metabolites; red spots represented upregulated differentially expressed metabolites; and gray represented non-differentially expressed metabolites. Heat map visualization (B) shows the overall situation of relative expression of 16 differential metabolites.
Source: Authors

namely, 2α- mannobiose, 3α-mannobiose, D-(+)-talose (anti), D-(+)-talose (syn), D-(+)-trehalose, D-fructose (Figure 3A–F). The MNB content of two kinds of group BBGs is obviously higher than that of SBGs group (Figure 3A and B). The contents of D-tal(anti) and D-tal (syn) in a gourd with different taste and flavor are distinct, and their content in BBGs are higher than SBGs (Figure 3C and D). The content of trehalose in BBGs is higher than SBGs (Figure 3E). In these battle gourds of this study, in BBGs, the relative content of D-fru is 5176 and 5037, respectively, whereas in SBGs, it is 4252, 4693, and 4335 (Table 1, Figure 3F).

In this research, there are two kinds of amino acids that are different in the metabolism of two groups of gourds with different flavors, namely 4-aminobutanoic acid and L-aspartic acid. The relative content of 4-aminobutanoic acid or L-Asp in BBGs is significantly more than the group SBGs (Figure 4A and B).

In this study, it was found that there were significant differences in the contents of butanedioic acid, phosphoric acid, propanedioic acid and shikimic acid in two different tastes and flavors of bottle gourd (Figure 5A-D). The content of these four acids in BBG cultivars is higher than that in SBG cultivars (Figure 5A-D).

In addition to the above-mentioned metabolites, there are significant differences in the content of various bottle gourds between the two groups, and there are also four metabolites, namely *myo*-Inositol, ethylene, ethylene glycol and propane. Similarly, BBGs have significantly larger levels of these four substances than SBGs (Figure 6A-D).

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) reveal differences in the metabolite profiles

In the PCA plot (Figure 7A), the duplicate samples were grouped together, indicating that the duplicate samples had similar metabolic profiles and that the entire analysis was stable and repeatable. Pairwise comparisons are carried out between BBG and SBG to identify the metabolites that are responsible for the taste differences. PLS-DA models are used to generate pairwise comparisons of the metabolite contributions. The predictability (Q2) and goodness of fit (R2X, R2Y) of the PLS-DA models were observed in the comparison between BBG and SBG (Q2=0.767, R2X=0.723, R2Y=0.981; Figure 7B).

Differential metabolic pathways between BBG and SBG

To obtain detailed pathway information, all metabolites

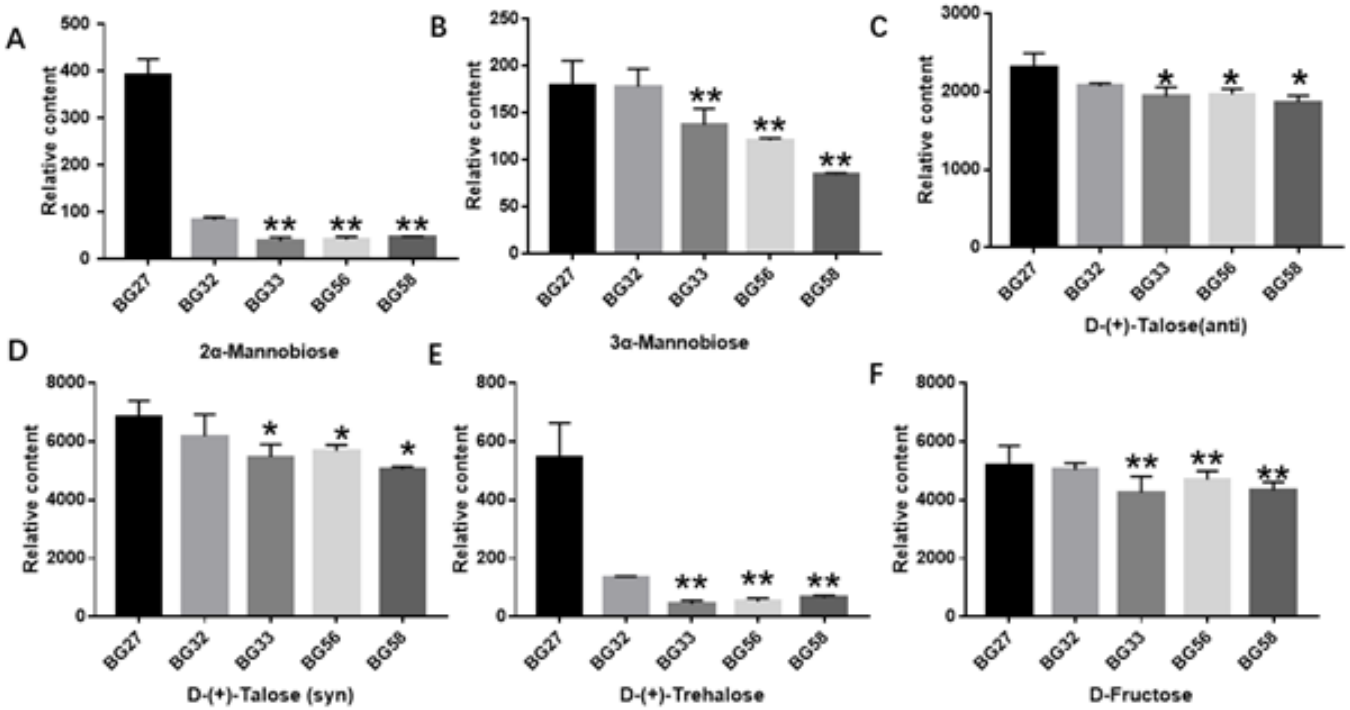

Figure 3. Saccharides content in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements. * and ** indicate a significant difference from that of BG27 at $p < 0.05$ and $p < 0.01$, respectively, by the Dunnett's test.
Source: Authors

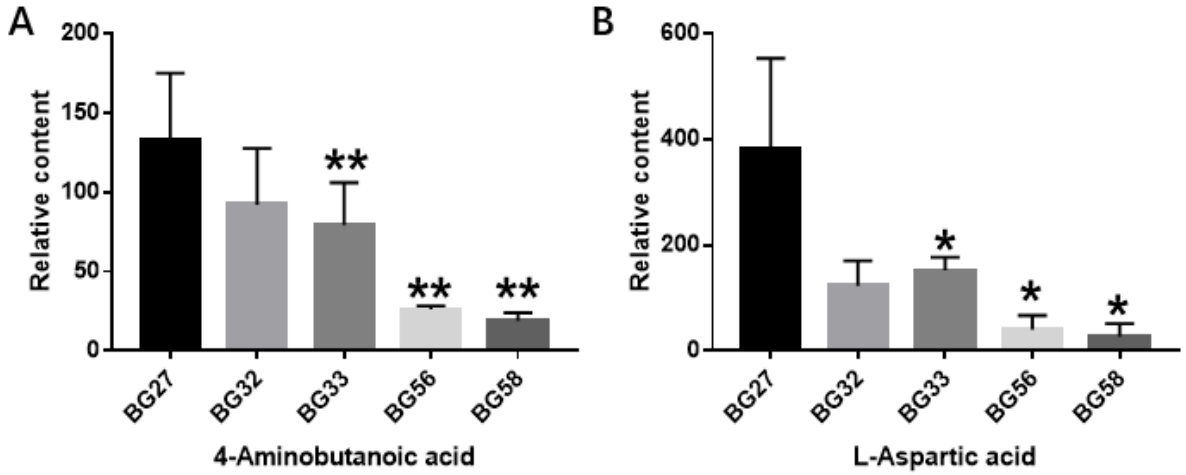

Figure 4. Amino acids content in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements. * and ** indicate a significant difference from that of BG27 at $p < 0.05$ and $p < 0.01$, respectively, by the Dunnett's test.
Source: Authors

and the differential metabolites were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) (Figure 8A and B). In the mapping of KEGG, there were 12 different metabolites involved in metabolisms, 1 involved in genetic information processing and 1 involved in environmental information processing (Figure 8A). The differential metabolites were mainly involved in beta-alanine metabolism, indole alkaloid biosynthesis, galactose metabolism, ect (Figure 8B).

Table 1. The relative content of 24 differential metabolites.

Compounds	The relative content														
	BG27			BG32			BG33			BG56			BG58		
2-αMannobiose	459.87	352.78	363.16	87.38	91.00	73.70	32.42	55.09	28.14	50.26	44.90	30.92	47.34	47.05	49.27
3-αMannobiose	200.69	186.40	148.10	198.84	172.34	162.70	156.40	131.58	123.52	123.09	118.84	120.39	84.18	82.79	86.01
4-Aminobutanoic acid	181.59	109.49	107.54	133.09	73.87	69.59	110.05	60.91	67.38	27.37	23.73	27.72	13.04	21.09	22.60
Butanedioic acid	52.94	49.10	42.59	123.90	56.07	55.39	38.38	37.90	29.60	45.74	39.86	39.58	19.05	28.10	25.49
D-(+)-Talose (anti)	2632.77	2300.85	1993.33	2134.59	2021.71	2060.50	2166.18	1754.75	1894.00	2105.26	1900.43	1890.99	1761.29	2029.89	1807.46
D-(+)-Talose (syn)	7807.93	6821.00	5884.16	5030.32	7568.70	5960.85	6328.20	5023.23	5057.73	5979.91	5299.36	5760.43	4924.17	5206.41	5036.75
D-(+)-Trehalose	677.82	448.57	508.57	132.17	139.13	136.96	41.22	57.68	35.15	65.88	48.78	41.60	58.44	69.41	71.15
D-Fructose	5640.66	5483.04	4405.97	5300.11	4915.11	4897.13	4886.84	3969.96	3903.63	5037.39	4518.13	4525.44	4148.26	4649.78	4209.55
Ethylene glycol	12.94	24.95	22.21	24.03	23.21	24.80	10.76	9.37	9.72	12.14	10.94	10.56	9.80	10.91	10.53
L-Aspartic acid	559.20	370.52	220.61	164.57	132.99	72.69	134.96	181.02	141.14	9.93	60.20	51.33	35.85	0.00	46.32
Myo-Inositol	1279.23	1005.94	1024.71	906.18	757.21	681.04	849.35	770.92	689.59	641.46	548.95	547.97	467.68	524.90	493.87
Oxalic acid	71.85	66.80	66.44	129.19	108.93	114.91	63.32	74.12	73.01	75.13	65.83	72.50	72.18	91.65	67.18
Phosphoric acid	74.15	45.08	63.60	56.06	42.03	38.87	34.72	32.06	21.90	20.32	18.38	18.03	14.55	15.43	18.34
Propane	129.42	86.12	112.60	23.26	26.66	23.45	8.93	18.78	10.16	15.29	13.94	10.66	18.62	16.91	18.57
Propanedioic acid	1995.95	1175.56	1681.84	1022.72	945.90	914.86	512.12	401.78	422.33	462.35	399.81	381.46	405.02	558.61	475.54
Shikimic acid	578.15	244.78	325.48	163.80	164.65	246.22	162.64	81.80	93.43	141.68	128.51	136.30	109.35	112.45	145.47
Ethylene	434.32	347.13	432.77	74.59	54.23	45.20	17.64	26.94	14.05	24.84	20.02	14.63	21.05	24.90	20.79

Source: Authors

DISCUSSION

Bottle gourd fruit varies in shape, size and flavor (Wu et al., 2017; Zhang et al., 2020). Previous studies have revealed the root causes of these differences at the genome and transcriptome levels (Konan et al., 2020). However, there is no research on the difference of metabonomics of gourd with different tastes. The metabolites of different gourd fruits are different. The metabolism of fruits with different tastes was different. GC-MS-based untargeted metabolomics of two groups of bottle gourd accessions yielded 16 differential metabolites. In previous reports, sugars were the main factor affecting sweetness (Cameron, 2011). The sweetness of the saccharides differs. Furthermore, the sweetness of some saccharides varies with the solution concentration, while the sweetness of others does not (Cameron, 2011). Mannobiose, composed of D-mannose units, is a nondigestible disaccharide and rich in Cucurbitaceae crops. MNB can modulate intestinal and systemic immune responses in mice and prevent LPS-induced immune suppression as well as directly stimulate innate immune mechanisms *in vitro* as a TLR4 agonist (Kovacs-Nolan et al., 2013). Therefore, the difference in MNB contained in bottle gourds with different tastes will play a certain role in cultivating bottle gourds with high MNB content. D-talose is a variety of rare monosaccharides, different from the exception of isomers such as D-glucose and D-fructose, which exist in great abundance and showed considerable growth inhibition upon the nematode (Sakoguchi et al., 2016). Trehalose is a nonreducing disaccharide with two glucose units linked in an α, α-1,1 configuration, widespread in plant kingdoms and its phosphorylation forms play a vital role in the integration of plant metabolism and development (Richards et al., 2002). Evidence showed that exogenously-supplied trehalose can make winter wheat better cope with heat stress (Luo et al., 2018). D-fru is one of the most common and also the sweetest monosaccharide, but it is not the substance with the highest

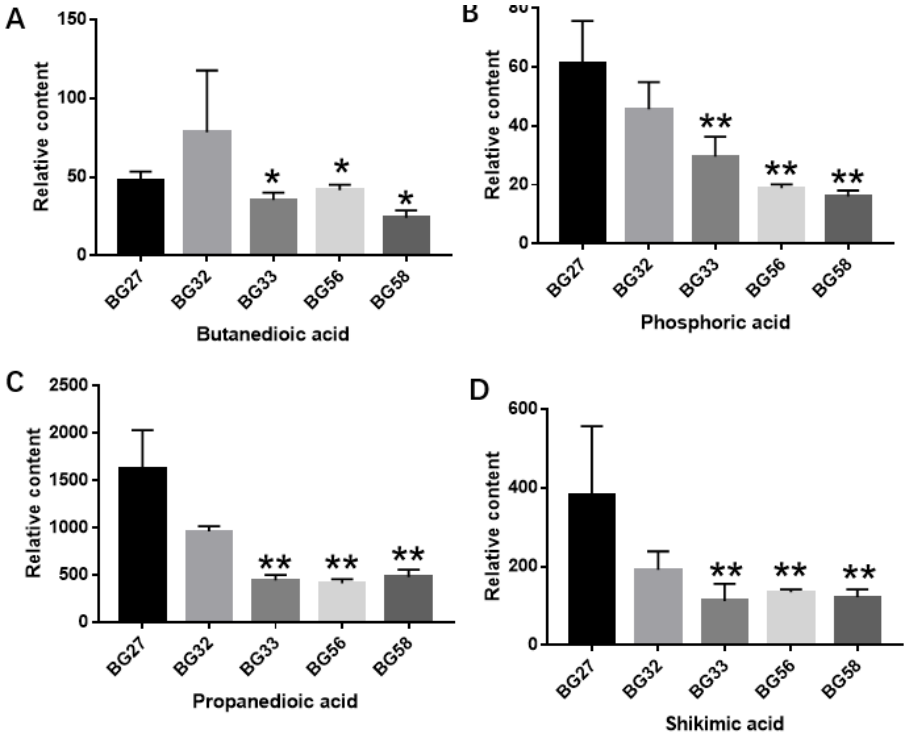

Figure 5. Organic acids and inorganic acidscontent in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements. * and ** indicate a significant difference from that of BG27 at $p < 0.05$ and $p < 0.01$, respectively, by the Dunnett's test.
Source: Authors

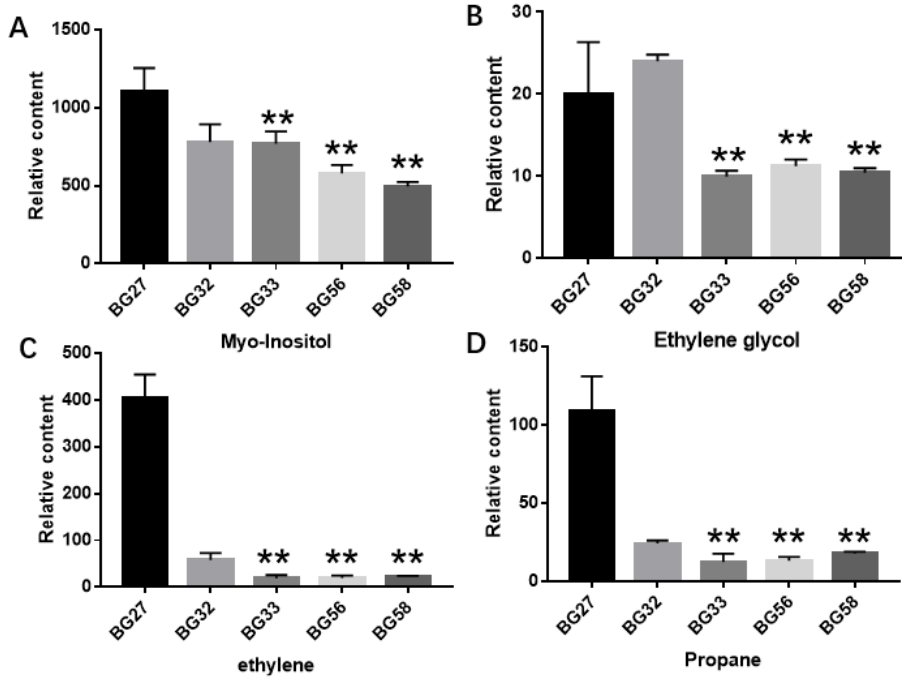

Figure 6. Other metabolites in the five bottle gourd cultivars. Data represent mean values ± SE of three independent measurements ** indicate a significant difference from that of BG27 at $p< 0.01$, by the Dunnett's test.
Source: Authors

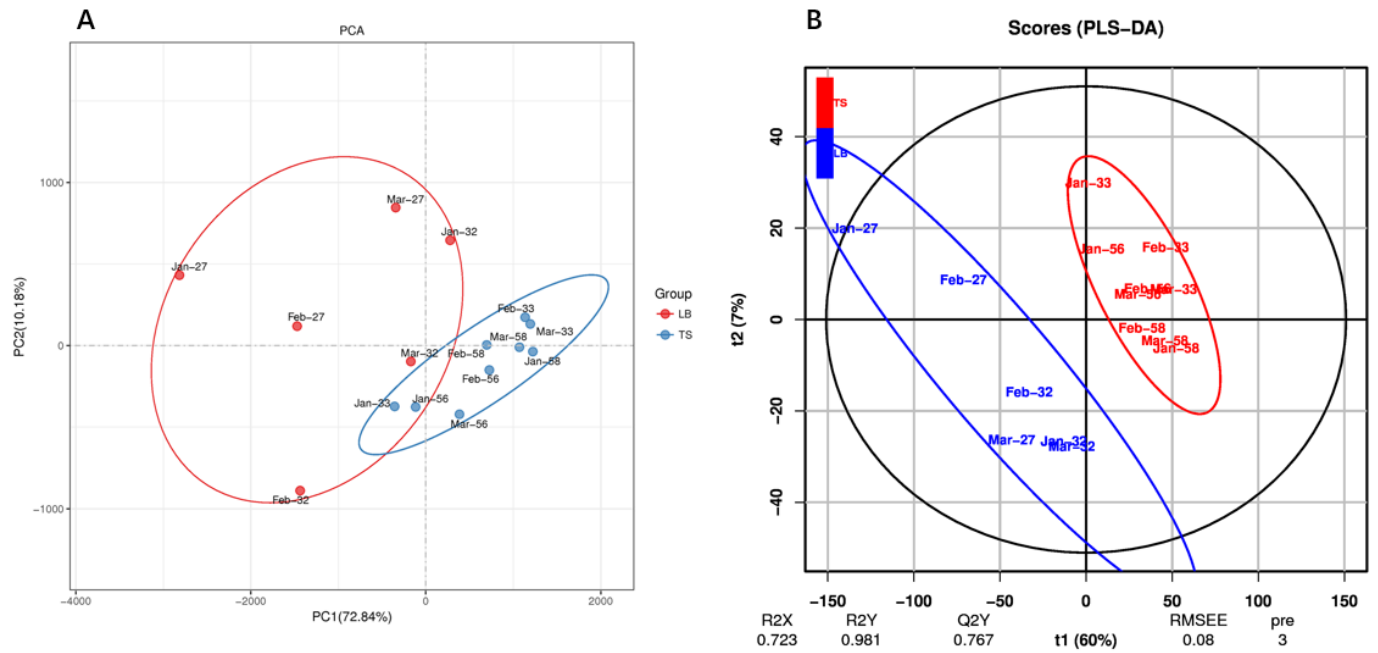

Figure 7. PCA and PLS-DA of the relative differences in secondary metabolites in BBGs and SBGs cultivars. BG27: Jan-27, Feb-27, Mar-7; BG32: Jan-32, Feb-32, Mar-32; BG33: Jan-33, Feb-33, Mar-33; BG56: Jan-56, Feb-56, Mar-56; BG58: Jan-58, Feb-58, Mar-58.
Source: Authors

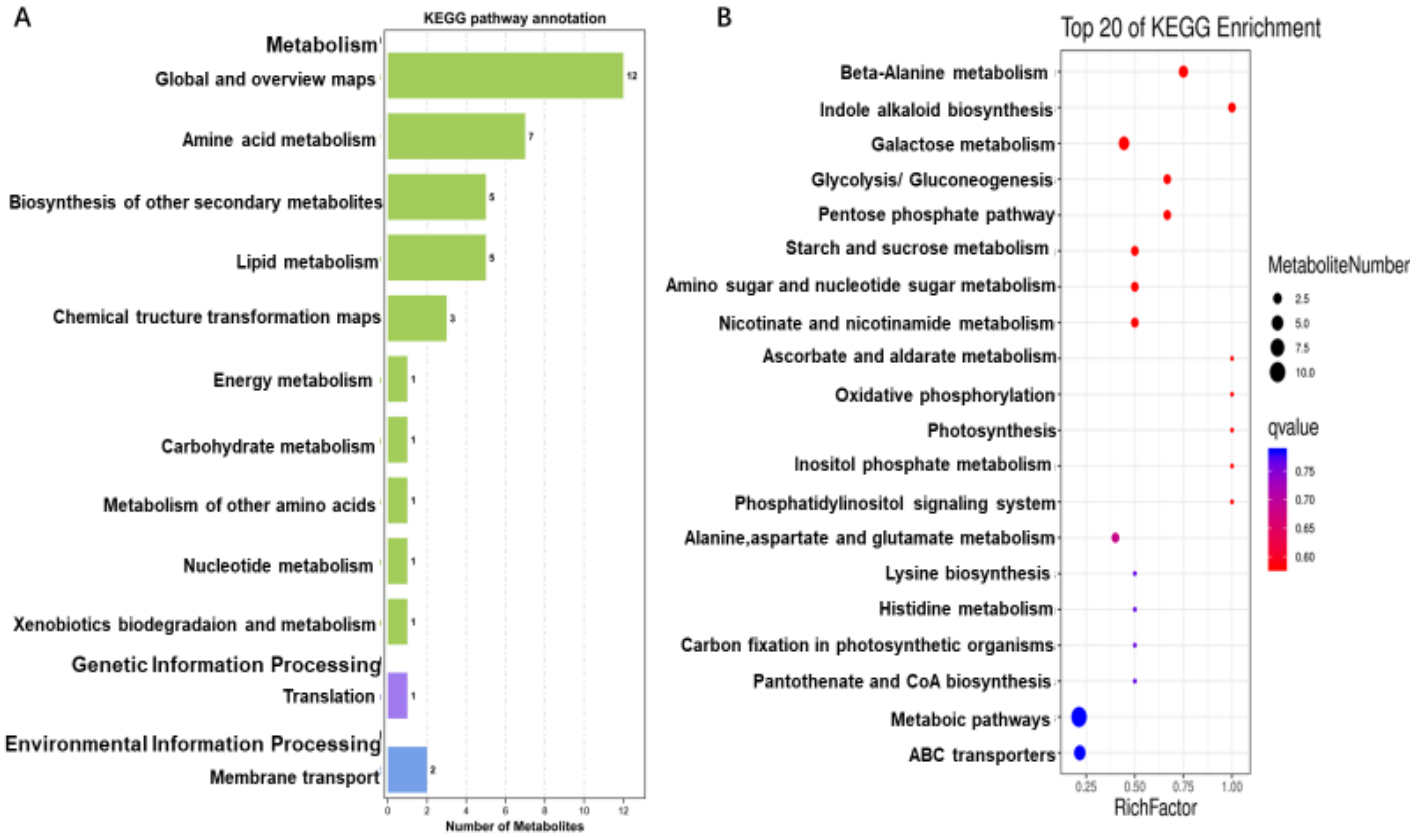

Figure 8. Enrichment of the differential metabolites to distinct KEGG pathways.
Source: Authors

sweetness in nature. For example, protein thaumatin, first isolated from the katemfe fruit, *Thaumatococcus daniellii*, is 1600 times sweeter than sucrose on a weight basis (Wel and Loeve, 1972). Therefore, it is probably not only sugar that determines the strong sweet taste of the gourd.

4-Aminobutanoic acid was also known as the name of gamma-Aminobutyric acid (GABA), was a non-proteinogenic amino acid and was first isolated from potato tubers in 1949 (Ramesh et al., 2017). In plants, GABA acts as a signal substance to regulate plant growth, development and response to stress. In mammals, its action has been mainly described in the nervous system, where GABA receptors regulate brain function and development. L-aspartic acid is one of the 20 types of amino acids that make up a protein's structural unit (Akcay et al., 2012). Free amino acids have been found as the main umami ingredients in many vegetables, especially free glutamate and free aspartate are the two main umami ingredients (Zhang et al., 2013). Previous data has shown that free glutamic acid is the major factor affecting umami taste in bottle gourds (Wu et al., 2017). However, our data found that there were significant differences in aspartic acid metabolism, but there was no significant difference in glutamic acid content in different flavors of bottle gourd.

Besides sugars and amino acids, sour ingredients have an important influence on the flavor of fruits. Whether the taste of the fruit is sour or sweet depends on the ratio of sweet substances to sour substances. Shikimic acid is widely found in higher plants, with high content in the fruit of Illicium verum, which has an anti-tumor effect and is also an important synthetic raw material of Tamiflu, an anti-influenza drug (Coleman, 2005). Myo-Inositol belongs to B vitamins and is the precursor of various biological functional compounds containing inositol phosphate and lipid. Inositol and phosphatidylinositol play a key role in many metabolic pathways, and if affected, they will have adverse effects on human health (Benvenga and Antonelli, 2016). Ethylene is a plant hormone that can regulate fruit ripening. Ethylene glycol and Propane also exist in the flesh of bottle gourd, but their unique roles are not clear.

Multivariate statistics are used to further assess the differences in metabolic profiles among the two gourd groups. PCA is one of the most widely used chemometric tools to reveal the internal structure of several variables by a few principal components and extract and rationalize information from any multivariate description of a biological system (Park et al., 2013). The results showed that the samples from the two cultivars were grouped into two areas in the plot, indicating that different flavors had relatively distinct metabolic profiles. Especially, the dots of a gourd with sweet taste are more concentrated than the dots of a gourd with slightly bitter taste, which indicates that the metabolic regulation leading to the sweet taste of the gourd is more consistent, while the metabolic regulation related to bitter taste is more complex. The plot suggests that the differences in metabolic profiles detected in our study correlated with fruit flavors. In the PLS-DA models, BBGs are clearly separated from SBGs, indicating major distinctions in the metabolic profiles between the different fruit tastes of bottle guards. For BG32 and SBGs, there was some overlap in PCA (Figure 7A), but a clear difference was obtained in the PLS-DA score plots (Figure 7B), indicating that the BG32 with a slightly bitter taste could be grouped from the guard with a sweet taste.

The roles of carbohydrates, and their metabolism, in plants have a vast influence on both plant growth and development. During the storage of coffee beans in the pod, the change of flavor is related to the metabolism of sugars and free amino acids in coffee beans (Hinneh et al., 2018). Previous evidence has found that, free glutamate content was a key factor conferring umami taste in the bottle gourd (Wu et al., 2017).

Conclusion

However, in the study, we found that there were differences in metabolic substances and metabolic pathways between the two different tastes of bottle gourd, which lay the foundation to discover the metabolic architecture of the sweety/bitter taste in bottle gourd and facilitate the breeding of new varieties with better taste and quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

This research was funded by the Shanghai Science and Technology Agriculture Promotion Project (2022-02-08-00-12-F01105). Thanks to the Instrumental analysis center, Shanghai Jiao Tong University for its instrument service.Thanks to the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences for providing the germplasm materials for the bottle gourd. Thanks to Guangzhou Gene Denovo Biotechnology Co., Ltd. for its Omicsmart cloud analysis platform.

REFERENCES

Akcay N, Bor M, Karabudak T, Ozdemir F, Turkan I (2012). Contribution of Gamma amino butyric acid (GABA) to salt stress responses of Nicotiana sylvestris CMSII mutant and wild type plants. Journal of Plant Physiology 169(5):452-458.

Benvenga S, Antonelli A (2016). Inositol(s) in thyroid function, growth and autoimmunity. Reviews in Endocrine and Metabolic Disorders 17(4):471-484.

Cameron AT (2011). The Relative Sweetnesses of Certain Susgars, Mixtures of Sugars, and Glycerol. Canadian Journal of Research 22(3):45-63.

Coleman WF (2005). Shikimic Acid. Journal of Chemical Education 82:656.
German JB, Watkins SM, Fay LB (2005). Metabolomics in Practice: Emerging Knowledge to Guide Future Dietetic Advice toward Individualized Health. Journal of the American Dietetic Association 105:1425-1432.
Healey RD, Lebhar H, Hornung S, Thordarson P,Marquis CP (2017). An improved process for the production of highly purified recombinant thaumatin tagged-variants. Food Chemistry 237:825-832.
Hinneh M, Semanhyia E, Van de Walle D, De Winne A, Tzompa-Sosa DA, Scalone GL, De Meulenaer B, Messens K, Van Durme J, Afoakwa EO, De Cooman L (2018). Assessing the influence of pod storage on sugar and free amino acid profiles and the implications on some Maillard reaction related flavor volatiles in Forastero cocoa beans. Food Research International 111:607-620.
Hobson GE, Bedford L (2015). The composition of cherry tomatoes and its relation to consumer acceptability. Journal of Horticultural Science 64(3):321-329.
Jaeger FH (2008). Simplified Plant Sample Preparation for use in Gas Chromatography-Mass Spectrometry (GC-MS) Based Metabolomic Profiling and Targeted Analyte Quantitation. Available at: https://repository.lib.ncsu.edu/handle/1840.16/2684
Kader AA, Stevens MA, Albright M,Morris LL (1978). Amino acid composition and flavor of fresh market tomatoes as influenced by fruit ripeness when harvested. Journal American Society for Horticultural Science 103(4):541-544.
Kafkas E, Koşar M, Türemiş N, Başer KHC (2006). Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. Food Chemistry 97(4):732-736.
Konan JA, Guyot R, Koffi KK, Vroh-Bi I,Zoro AIB (2020). Molecular confirmation of varietal status in bottle gourd (*Lagenaria siceraria*) using genotyping-by-sequencing. Genome 63(11):535-545.
Kovacs-Nolan J, Kanatani H, Nakamura A, Ibuki M,Mine Y (2013). beta-1,4-mannobiose stimulates innate immune responses and induces TLR4-dependent activation of mouse macrophages but reduces severity of inflammation during endotoxemia in mice. Journal of Nutrition 143(3):384-391.
Kurihara K (2009). Glutamate: from discovery as a food flavor to role as a basic taste (umami). American Journal of Clinical Nutrition 90(3):719S-722S.
Luo Y, Wang W, Fan YZ, Gao YM, Wang D (2018). Exogenously-Supplied Trehalose Provides Better Protection for D1 Protein in Winter Wheat under Heat Stress. Russian Journal of Plant Physiology 65(1):115-122.
Park S-Y, Lim S-H, Ha S-H, Yeo Y, Park WT, Kwon DY, Park SU,Kim JK (2013). Metabolite Profiling Approach Reveals the Interface of Primary and Secondary Metabolism in Colored Cauliflowers (*Brassica oleracea* L. ssp. botrytis). Journal of Agricultural and Food Chemistry 61(28):6999-7007.
Ramesh SA, Tyerman SD, Gilliham M, Xu B (2017). γ-Aminobutyric acid (GABA) signalling in plants. Cellular and Molecular Life Sciences 74(9):1577-1603.
Richards AB, Krakowka S, Dexter LB, Schmid H, Wolterbeek APM, Waalkens-Berendsen DH, Shigoyuki A,Kurimoto M (2002). Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. Food and Chemical Toxicology 40(7):871-898.
Robert PW, Franco F (2018) Organic Acids in Fruits: Metabolism, Functions and Contents. John Wiley & Sons, Inc, Perugia.
Sakoguchi H, Yoshihara A, Izumori K, Sato M (2016). Screening of biologically active monosaccharides: growth inhibitory effects of D-allose, D-talose, and L-idose against the nematode Caenorhabditis elegans. Bioscience,Biotechnology and Biochemistry 80(6):1058-1061.
Sari HY (2003). Effect of different rootstock on plant growth, yield and quality of watermelon. Animal Production Science 43(10):1269-1274.
Verma A, Jaiswal S (2015). Bottle gourd (*Lagenaria siceraria*) juice poisoning. World Journal of Emergency Medicine 6(4):308.
Wang A, Li R, Ren L, Gao X, Zhang Y, Ma Z, Ma D,Luo Y (2018). A comparative metabolomics study of flavonoids in sweet potato with different flesh colors (*Ipomoea batatas* (L.) Lam). Food Chemistry 260:124-134.
Weckwerth W, Wenzel K, Fiehn O (2004). Process for the integrated extraction, identification and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks. Proteomics 4(1):78-83.
Wel HVD, Loeve K (1972). Isolation and Characterization of Thaumatin I and II, the Sweet -Tasting Proteins from Thaumatococcus daniellii Benth. European Journal of Biochemistry 31(2):221-225.
Wu X, Xu P, Wu X, Wang B, Lu Z,Li G (2017). Genome-wide association analysis of free glutamate content, a key factor conferring umami taste in the bottle gourd [*Lagenaria siceraria* (Mol.) Standl.]. Scientia Horticulturae 225:795-801.
Xu P, Wu X, Jie L, Wang B, Li G (2011). Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. BMC Genomics 12:467.
Zhang H, Tan J, Zhang M, Huang S, Chen X (2020). Comparative Transcriptomic Analysis of Two Bottle Gourd Accessions Differing in Fruit Size. Genes 11(4):359.
Zhang Y, Venkitasamy C, Pan Z, Wang W (2013). Recent developments on umami ingredients of edible mushrooms – A review. Trends in Food Science and Technology 33(2):78-92.

Supplementary Table S1

Table S1 A list of 155 compounds identified in bottle gourd flesh

Compounds	The Relative Content															control_mean	case_mean	log2_FC(case_mean/control_mean)	Pvalue	fdr	vip
	BG27			BG32			BG33			BG56			BG58								
1,2,3-Butanetriol	2.55	1.91	1.80	1.05	0.93	1.03	1.62	1.48	1.23	0.00	0.59	0.00	0.00	0.00	0.00	1.54	0.55	(1.50)	0.02	0.04	0.18
1,2-Butanediol	0.00	33.42	39.08	0.00	6.96	7.89	0.00	6.76	0.00	6.42	5.74	4.59	4.54	4.67	4.66	14.56	4.15	(1.81)	0.09	0.15	0.63
1,2-Ethenediol	5.52	6.25	5.00	5.74	5.11	4.96	4.44	3.45	3.28	4.28	4.18	3.66	2.93	3.52	3.75	5.43	3.72	(0.55)	0.00	0.00	0.30
1-Monopalmitin	0.38	0.14	0.21	0.29	0.09	0.15	0.17	0.10	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.21	0.04	(2.50)	0.00	0.01	0.08
1-O-trans-p-Coumaroylglycerol	0.00	0.00	0.00	1.22	2.46	2.63	0.00	0.67	0.33	0.00	0.41	0.00	2.79	2.99	2.66	1.05	1.09	0.06	0.95	0.96	0.23
1H-Indole-2,3-dione	8.30	5.83	7.45	4.05	0.00	0.00	1.77	3.08	2.01	0.00	0.00	0.00	0.00	1.27	1.30	4.27	1.05	(2.03)	0.02	0.05	0.27
2'-Hydroxy-6'-methyl-3,4-methylenedioxychalcone	5.31	4.54	0.00	1.18	0.00	0.00	0.00	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.84	0.08	(4.51)	0.05	0.09	0.24
2,3,4-Trihydroxybutyric acid	4.78	3.99	3.52	3.91	2.69	2.75	2.49	3.12	2.21	2.29	1.88	1.72	1.14	1.37	1.23	3.61	1.94	(0.89)	0.00	0.00	0.25
methylphosphonofluoridate	0.28	0.00	0.00	0.39	0.26	0.42	0.00	0.39	0.27	0.22	0.20	0.00	0.21	0.28	0.66	0.23	0.25	0.13	0.83	0.88	0.05
2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa	1.52	0.00	0.00	3.13	2.18	2.00	0.00	0.00	2.07	0.00	0.89	0.00	0.00	1.71	4.25	1.47	0.99	(0.57)	0.52	0.61	0.22
2,4-Dimethyl-3-pentanol	4.40	3.68	3.14	0.00	0.00	0.00	0.00	0.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.87	0.10	(4.26)	0.02	0.05	0.22
benzonitrile	4.30	3.76	4.43	0.77	1.39	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.55	0.00	(11.32)	0.00	0.00	0.28
2-αMannobiose	459.87	352.78	363.16	87.38	91.00	73.70	32.42	55.09	28.14	50.26	44.90	30.92	47.34	47.05	49.27	237.98	42.82	(2.47)	0.00	0.02	2.33
2-Butene-1,4-diol	0.93	0.89	0.85	0.96	0.91	0.90	0.79	0.68	0.50	0.81	0.60	0.68	0.52	0.71	0.62	0.91	0.66	(0.47)	0.00	0.00	0.11
2-Butenedioic acid	6.41	3.86	2.67	14.53	4.49	3.50	3.87	3.04	2.65	2.42	2.20	2.13	1.36	1.41	1.68	5.91	2.31	(1.36)	0.03	0.06	0.46
propane	1.48	1.38	1.26	1.29	1.30	1.44	1.25	1.24	1.15	1.38	1.28	1.26	1.26	1.55	1.32	1.36	1.30	(0.06)	0.32	0.42	0.04
2-Hydroxychalcone	3.07	0.00	2.57	5.00	2.62	3.32	0.00	1.20	3.09	1.70	0.00	0.00	0.00	2.59	7.04	2.76	1.74	(0.67)	0.36	0.48	0.28
2-O-Glycerol-d-galactopyranoside	17.25	7.25	11.19	8.05	3.31	4.50	7.75	1.85	3.01	1.77	1.47	1.20	0.61	0.00	0.00	8.59	1.96	(2.13)	0.00	0.02	0.42
3,5-Dihydroxyflavone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.22	0.00	0.33	0.00	0.00	0.09	6.46	0.14	0.22	0.08
3-αMannobiose	14.92	10.40	9.46	6.32	5.88	5.43	6.57	6.46	4.87	4.84	4.42	3.99	3.79	4.12	3.83	8.74	4.77	(0.87)	0.01	0.03	0.34
3-Amino-2-piperidone	2.82	1.71	0.00	0.00	0.00	0.00	0.00	1.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.13	(2.54)	0.17	0.25	0.18
3-Methyl-1,2-bis(trimethylsilyl)butane	14.22	11.64	12.13	11.29	14.95	13.07	2.08	2.33	1.58	1.89	2.68	2.54	1.51	1.11	1.70	12.89	1.94	(2.73)	0.00	0.00	0.91
3-Oxaoct-4-en-2-imine	116.01	106.64	112.96	130.14	123.90	144.49	132.60	137.93	126.75	130.72	112.61	120.55	135.43	155.87	131.77	122.36	131.58	0.10	0.19	0.27	0.39
3-Pyridinol	7.37	7.77	7.60	7.86	7.09	7.77	7.60	6.70	6.69	8.10	6.83	7.32	7.34	8.71	7.45	7.57	7.42	(0.03)	0.60	0.67	0.07
4-Aminobutanoic acid	181.59	109.49	107.54	133.09	73.87	69.59	110.05	60.91	67.38	27.37	23.73	27.72	13.04	21.09	22.60	112.53	41.54	(1.44)	0.00	0.01	1.49
4-Ethoxy-7-methoxycoumarin	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.22	0.25	0.00	0.00	0.00	0.05	0.08	0.55	0.71	0.78	0.04
4-Hydroxybenzyl alcohol	3.09	2.08	2.92	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	0.00	(10.45)	0.01	0.03	0.20
4-Methyl-1,2-bis(trimethylsilyloxy)pentane	0.98	0.58	0.71	0.66	0.52	0.40	0.40	0.45	0.31	0.21	0.21	0.41	0.00	0.18	0.00	0.64	0.24	(1.41)	0.00	0.01	0.12
5-Iodouridine	0.00	9.64	7.60	6.33	5.51	0.00	5.41	4.55	3.95	0.00	4.55	0.00	4.17	0.00	0.00	4.85	2.51	(0.95)	0.18	0.26	0.42
7-Diethylaminocoumarin	1.41	1.33	1.86	0.73	0.49	0.31	0.15	0.47	0.14	0.29	0.35	0.00	0.21	0.00	0.18	1.02	0.20	(2.36)	0.00	0.01	0.17
7-Hydroxy-4-(methoxymethyl)coumarin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.08	0.05	0.00	0.00	0.00	0.00	0.00	0.02	4.34	0.14	0.22	0.03
7-Nitroindazole	4.89	0.00	2.86	2.21	2.24	2.01	0.00	0.00	1.84	1.83	0.00	0.00	0.00	0.00	0.00	2.37	0.41	(2.54)	0.01	0.02	0.25
D-Galactofuranoside	25.41	21.30	16.86	17.41	17.84	0.00	18.37	13.66	14.00	16.09	14.38	13.83	11.84	15.28	13.31	16.47	14.53	(0.18)	0.52	0.61	0.56
D-Glucopyranuronic acid	22.61	17.85	14.31	13.91	11.02	7.87	7.79	7.98	5.78	6.44	5.33	4.64	3.56	3.63	3.54	14.59	5.41	(1.43)	0.00	0.00	0.55
Gentiobiose	7.09	4.14	0.00	0.00	0.28	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.02	(6.71)	0.08	0.13	0.30
Linolenic acid	3.58	2.44	4.09	5.05	2.87	2.96	3.76	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.50	0.65	(2.43)	0.00	0.00	0.41
Adenosine	7.28	4.60	5.41	8.29	4.88	4.52	3.37	4.41	2.84	1.96	2.03	1.57	0.98	1.17	1.06	5.83	2.16	(1.43)	0.00	0.00	0.47
Alanine	3.41	3.41	3.38	2.09	2.43	2.45	2.44	1.95	1.40	1.62	2.75	0.00	1.79	0.69	0.00	2.86	1.40	(1.03)	0.01	0.02	0.22
Allyldimethyl(prop-1-ynyl)silane	1.41	0.00	1.06	2.46	1.75	0.00	0.00	1.40	1.57	1.03	1.02	0.80	0.00	0.00	3.96	1.11	1.09	(0.03)	0.97	0.97	0.07
Arabinitol	6.53	4.83	6.12	6.17	5.12	4.58	3.82	4.67	3.54	2.98	2.83	2.50	1.92	2.27	2.02	5.56	2.95	(0.91)	0.00	0.00	0.38
Arabinofuranose	0.00	7.52	6.79	9.28	7.52	6.05	3.31	3.28	2.41	4.14	4.24	0.00	3.47	0.00	3.57	6.19	2.71	(1.19)	0.02	0.04	0.72
Asparagine	10.87	5.81	33.23	2.72	0.00	0.00	7.34	18.91	15.10	0.00	0.00	0.00	0.00	0.00	0.00	8.77	4.59	(0.93)	0.43	0.53	0.21
Boric acid	2.67	1.80	1.61	0.00	1.00	1.14	4.44	1.43	2.43	3.41	1.54	1.73	3.85	3.69	4.77	1.37	3.03	1.15	0.02	0.04	0.45

Supplementary Table S1. Cont'd

D-Butanal	7.01	6.07	5.51	5.37	3.49	3.66	4.89	2.93	3.03	3.11	2.05	2.15	1.48	1.84	2.12	5.19	2.62	(0.98)	0.00	0.01	0.28
L-Butanal	2.75	2.43	1.94	0.00	1.39	1.38	1.30	0.60	0.83	1.62	1.38	1.38	0.94	1.13	1.25	1.65	1.16	(0.51)	0.18	0.26	0.18
Butanedioic acid	52.94	49.10	42.59	123.90	56.07	55.39	38.38	37.90	29.60	45.74	39.86	39.58	19.05	28.10	25.49	63.33	33.74	(0.91)	0.01	0.04	1.54
Butanoic acid	6.14	4.48	3.35	0.41	0.00	0.00	1.88	1.47	1.33	2.07	1.83	1.44	1.08	1.08	1.13	2.40	1.48	(0.69)	0.31	0.42	0.34
Cadaverine	0.42	0.33	0.00	0.00	0.29	0.33	0.00	0.00	0.48	0.48	0.43	0.00	0.47	0.53	0.00	0.23	0.27	0.22	0.76	0.82	0.05
Caffeic acid	1.74	1.44	1.43	0.81	0.98	0.98	1.81	1.14	1.18	1.61	1.35	1.27	0.82	1.65	1.35	1.23	1.35	0.14	0.49	0.58	0.22
Cephalothin	0.00	86.32	0.00	87.81	78.60	77.66	0.00	0.00	70.42	0.00	0.00	0.00	69.75	69.47	72.78	55.06	31.38	(0.81)	0.28	0.38	2.00
Chizo-Inositol	0.00	1.26	1.19	1.81	0.00	0.00	0.79	1.14	1.03	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.33	(1.11)	0.28	0.38	0.18
Citric acid	33.23	21.74	27.21	19.46	15.69	16.99	15.19	12.83	9.69	10.51	9.20	8.69	7.12	8.41	7.44	22.38	9.90	(1.18)	0.00	0.00	0.65
Cyclohexanol	0.33	0.00	0.00	0.47	0.25	0.39	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.34	0.62	0.24	0.16	(0.55)	0.55	0.63	0.07
D-(+)-Talofuranose	3.06	2.38	2.11	1.62	0.00	0.00	0.00	2.01	1.56	0.00	0.00	0.00	0.00	0.00	0.00	1.53	0.40	(1.95)	0.05	0.10	0.16
D-(+)-Talose	2632.77	2300.85	1993.33	2134.59	2021.71	2060.50	2166.18	1754.75	1894.00	2105.26	1900.43	1890.99	1761.29	2029.89	1807.46	2190.62	1923.36	(0.19)	0.02	0.04	2.98
D-(+)-Talose	7807.93	6821.00	5884.16	5030.32	7568.70	5960.85	6328.20	5023.23	5057.73	5979.91	5299.36	5760.43	4924.17	5206.41	5036.75	6512.16	5401.80	(0.27)	0.02	0.04	5.51
D-(+)-Trehalose	677.82	448.57	508.57	132.17	139.13	136.96	41.22	57.68	35.15	65.88	48.78	41.60	58.44	69.41	71.15	340.54	54.37	(2.65)	0.00	0.01	2.87
D-(-)-Fructose	2.49	2.74	2.01	12.35	0.59	6.82	0.80	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.50	0.23	(4.30)	0.01	0.03	0.68
D-(-)-Ribofuranose	206.66	190.94	159.09	94.18	103.90	110.26	198.52	125.38	140.83	162.80	155.67	149.16	141.28	151.44	161.56	144.17	154.07	0.10	0.59	0.66	2.25
D-Allose	9.66	10.40	8.73	11.76	9.52	10.42	10.41	9.33	8.57	10.56	8.88	8.40	8.35	9.13	8.44	10.08	9.12	(0.14)	0.07	0.12	0.21
D-Arabinose	74.58	60.15	44.75	87.30	75.65	71.54	75.13	55.14	56.78	90.77	73.56	73.61	40.24	46.22	47.37	69.00	62.09	(0.15)	0.43	0.53	0.34
D-Fructose	5640.66	5483.04	4405.97	5300.11	4915.11	4897.13	4886.84	3969.96	3903.63	5037.39	4518.13	4525.44	4148.26	4649.78	4209.55	5107.00	4427.66	(0.21)	0.01	0.03	4.15
D-Galactose	4.77	13.16	2.40	6.96	10.49	20.45	4.05	5.64	3.71	8.08	6.56	0.00	5.63	4.71	6.33	9.71	4.97	(0.97)	0.06	0.12	0.69
D-Glucitol	3.01	3.04	2.41	3.84	3.34	2.47	1.76	1.84	1.82	1.92	1.52	1.83	1.29	0.97	1.45	3.02	1.60	(0.92)	0.00	0.00	0.33
D-Glucose	0.00	0.00	274.44	0.00	0.00	116.61	0.00	100.26	0.00	94.71	0.00	90.53	0.00	99.92	91.22	65.17	52.96	(0.30)	0.78	0.82	1.14
D-Mannose	11.40	11.48	9.43	0.00	6.71	0.00	25.51	15.45	16.94	5.30	5.73	13.57	7.57	3.09	2.37	6.50	10.61	0.71	0.28	0.38	0.73
D-Ribose	3.03	3.83	1.77	3.03	3.41	4.50	3.02	2.93	1.98	2.71	1.61	1.68	2.23	3.27	3.43	3.26	2.54	(0.36)	0.10	0.17	0.19
D-Threitol	3.93	3.01	3.11	3.28	2.71	2.41	2.44	2.57	2.09	1.65	1.44	1.35	1.13	1.69	1.23	3.07	1.73	(0.83)	0.00	0.00	0.24
D-Xylose	0.00	1.38	0.76	0.00	0.00	0.00	0.00	0.64	0.42	0.00	0.57	0.00	0.80	0.00	0.00	0.36	0.27	(0.40)	0.72	0.79	0.06
DL-Homocysteine	0.00	0.00	0.00	2.65	0.63	0.00	0.92	2.25	0.94	0.59	0.31	0.28	0.37	0.41	0.00	0.55	0.68	0.30	0.78	0.82	0.14
DL-Phenylalanine, TMS derivative	1.41	3.45	15.58	11.48	0.00	0.00	18.58	14.24	11.55	5.15	5.05	4.20	5.49	0.00	3.05	5.32	7.48	0.49	0.52	0.61	0.21
Decanedioic acid	0.29	0.00	0.41	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	(7.49)	0.02	0.04	0.08
Demexiptiline	3.38	1.83	1.83	0.00	0.00	0.00	1.16	0.96	0.94	0.00	0.45	0.00	0.34	0.00	0.00	1.17	0.43	(1.45)	0.16	0.24	0.20
Disiloxane	12.44	6.40	6.92	0.00	2.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.74	0.00	(12.21)	0.01	0.03	0.38
Dopamine	0.30	0.89	0.92	0.00	1.42	0.00	0.00	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.59	0.05	(3.49)	0.02	0.04	0.14
Erythritol	7.98	6.41	5.77	3.89	3.29	3.38	4.73	4.60	3.54	4.86	4.00	3.74	3.43	3.60	3.34	5.12	3.98	(0.36)	0.11	0.18	0.26
Ethanesulfonic acid	0.53	0.39	0.43	1.08	0.44	0.57	0.57	0.85	0.58	0.48	0.30	0.32	0.34	0.30	0.33	0.57	0.45	(0.34)	0.31	0.42	0.11
Ethanolamine	28.58	18.14	23.64	34.62	17.70	15.87	22.95	17.40	15.91	9.64	12.90	10.72	6.52	6.40	8.60	23.09	12.34	(0.90)	0.01	0.02	0.67
Ethyl D-glucopyranoside	4.39	4.13	3.21	5.65	4.07	4.58	4.25	5.24	3.68	4.98	3.37	3.52	3.45	4.11	2.81	4.34	3.93	(0.14)	0.35	0.46	0.13
Ethylene glycol	12.94	24.95	22.21	24.03	23.21	24.80	10.76	9.37	9.72	12.14	10.94	10.56	9.80	10.91	10.53	22.02	10.53	(1.07)	0.00	0.00	1.16
Fructofuranoside	4.70	2.97	3.09	3.54	3.51	3.22	2.07	1.46	1.40	1.49	1.46	1.22	0.95	0.87	0.97	3.50	1.32	(1.41)	0.00	0.00	0.33
Galactitol	8.29	7.27	6.29	6.82	5.93	7.10	7.08	6.84	5.49	6.60	5.48	5.11	5.58	5.84	5.14	6.95	5.90	(0.23)	0.02	0.05	0.17
Galactose oxime	21.70	18.22	8.72	14.11	13.82	10.82	20.94	10.44	12.53	13.74	16.16	14.95	8.27	8.28	13.08	14.57	13.15	(0.15)	0.54	0.63	0.48
Glipizide	4.92	4.15	4.79	14.67	1.76	3.61	9.49	45.35	10.75	4.92	2.34	2.07	7.67	9.51	2.23	5.65	10.48	0.89	0.42	0.53	0.41
Glucoheptonic acid	0.00	3.91	0.00	3.88	0.00	3.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	0.00	(10.86)	0.02	0.04	0.45
Glucopyranose	0.00	0.14	0.00	0.00	0.17	0.00	0.16	0.00	0.19	0.19	0.14	0.00	0.21	0.00	0.00	0.05	0.10	0.93	0.34	0.45	0.04
Glucose	189.14	158.38	143.57	91.27	93.78	104.40	180.87	114.74	139.14	164.57	142.53	136.77	133.96	158.85	143.54	130.09	146.11	0.17	0.31	0.42	2.36
Gly-Asp-Lys	3.15	6.19	0.00	0.00	0.00	0.00	0.00	0.00	12.46	0.00	0.00	0.00	0.00	0.00	0.00	1.56	1.38	(0.17)	0.93	0.95	0.15
Glyceric acid	55.70	37.11	38.70	39.29	29.00	32.45	27.59	21.36	17.20	25.55	20.50	21.21	15.84	21.06	18.96	38.71	21.03	(0.88)	0.00	0.00	0.77
Glycerol	69.37	44.64	52.05	51.73	34.70	35.14	38.82	29.96	30.09	25.86	23.84	22.80	21.73	24.46	22.12	47.94	26.63	(0.85)	0.00	0.00	0.82
Glyceryl-glycoside TMS ether	5.82	3.50	3.73	4.18	3.12	3.03	3.59	2.04	1.65	1.82	1.48	1.66	0.95	1.33	1.45	3.90	1.78	(1.13)	0.00	0.00	0.26
Glycine	24.64	20.91	22.92	21.88	14.52	13.37	23.31	28.00	19.92	24.19	27.45	25.34	19.41	22.17	20.86	19.71	23.40	0.25	0.09	0.14	0.68
Glycolic acid	28.76	16.49	19.14	3.66	3.00	1.58	3.00	2.05	2.00	2.28	1.92	2.08	1.98	2.62	1.84	12.11	2.20	(2.46)	0.02	0.04	0.56

Supplementary Table S1. Cont'd

Hydracrylic acid	4.28	4.31	3.58	4.25	3.55	3.60	3.10	3.04	2.32	2.25	2.80	3.05	2.97	3.23	2.41	3.93	2.80	(0.49)	0.00	0.00	0.24
Hydroxylamine	1.00	3.94	0.00	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.82	0.06	(3.74)	0.17	0.25	0.14
Ile-Trp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.10	0.13	0.12	0.00	0.00	0.00	0.05	5.71	0.06	0.12	0.05
Indoramin	0.00	0.00	1.17	1.51	0.00	0.00	0.00	0.48	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.07	(2.60)	0.14	0.22	0.19
Isocitric acid lactone	1.97	0.00	1.53	1.29	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.04	(4.31)	0.02	0.05	0.14
Kenpaullone	0.00	0.00	12.88	2.55	0.00	0.00	8.16	10.27	9.46	0.00	0.00	0.00	0.00	5.95	0.00	2.57	3.76	0.55	0.65	0.72	0.10
L-(+)-Threose	1.53	2.62	3.01	2.91	2.46	2.63	3.57	3.35	3.07	3.54	3.15	2.32	3.56	4.43	2.70	2.53	3.30	0.39	0.02	0.05	0.14
L-5-Oxoproline	354.46	347.75	609.11	550.95	264.70	210.44	847.06	1050.33	756.68	318.75	436.57	365.79	404.51	449.34	451.62	389.57	564.52	0.54	0.16	0.24	2.43
L-Alanine	52.94	45.41	30.31	40.35	28.06	30.96	32.10	31.99	26.12	23.77	14.08	17.20	13.08	21.33	27.53	38.00	23.02	(0.72)	0.00	0.02	0.68
L-Aspartic acid	17.62	17.19	25.50	21.48	14.00	13.46	15.00	12.69	12.05	5.55	5.04	3.49	3.27	2.07	1.95	18.21	6.79	(1.42)	0.00	0.00	0.83
L-Glutamic acid	1.63	1.33	1.43	0.88	1.18	0.00	0.00	1.32	0.00	0.00	0.55	0.38	0.55	0.00	0.34	1.08	0.35	(1.62)	0.02	0.04	0.15
L-Glutamine	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.39	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.06	0.13	0.94	0.96	0.07
L-Isoleucine	1.35	0.00	1.40	2.78	0.93	0.00	4.23	4.28	3.14	3.35	4.03	3.54	1.97	2.72	2.95	1.08	3.36	1.64	0.00	0.00	0.40
L-Lysine	18.27	14.57	21.57	16.57	8.65	8.14	24.01	21.65	17.29	13.45	16.95	17.40	10.28	9.65	12.92	14.63	15.95	0.13	0.63	0.70	0.42
L-Ornithine,	0.00	0.00	0.00	0.00	0.00	0.00	0.85	6.62	2.33	0.00	1.63	0.00	0.00	0.28	2.61	0.00	1.59	10.64	0.10	0.16	0.22
L-Phenylalanine	5.80	5.47	6.82	5.81	3.77	2.75	11.55	12.93	9.72	3.94	5.76	5.14	4.65	4.88	5.35	5.07	7.10	0.49	0.19	0.27	0.34
L-Rhamnose	4.71	3.69	3.01	3.18	2.49	2.38	2.07	1.77	1.53	1.77	1.52	1.31	1.20	1.35	1.41	3.24	1.55	(1.07)	0.00	0.00	0.24
L-Serine	32.29	23.94	31.73	52.80	18.80	12.09	46.51	53.76	38.18	24.22	37.89	31.11	19.33	26.56	25.56	28.61	33.68	0.24	0.45	0.55	0.43
L-Threonine	14.00	10.91	13.89	11.51	5.18	4.66	15.36	16.97	13.64	7.71	9.36	8.67	7.34	9.22	8.44	10.03	10.75	0.10	0.73	0.79	0.35
L-Threonine	0.00	0.17	0.08	0.00	0.17	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.02	(2.21)	0.12	0.19	0.04
L-Tyrosine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.63	16.37	0.00	11.64	13.98	0.00	6.74	12.72	0.07	0.12	0.55
L-Valine	7.18	6.02	5.24	5.18	3.93	3.84	3.05	3.80	3.20	4.97	2.75	4.15	3.25	5.68	4.24	5.23	3.90	(0.42)	0.04	0.07	0.19
L-Valine	3.53	3.51	3.56	5.24	2.40	2.06	7.17	6.63	5.56	5.03	5.81	5.39	3.59	3.93	4.17	3.38	5.25	0.63	0.01	0.03	0.36
Lactic Acid	32.79	69.89	26.51	17.46	13.43	22.19	33.20	17.02	18.42	22.18	19.27	22.41	16.27	16.49	17.11	30.38	20.26	(0.58)	0.18	0.26	0.53
Leu-Gly	0.00	0.00	1.23	3.76	1.16	0.00	4.16	4.27	3.20	2.62	3.28	2.80	0.00	1.95	1.94	1.02	2.69	1.39	0.04	0.07	0.23
Linoelaidic acid	2.19	1.87	2.50	3.43	2.14	2.44	3.36	1.64	1.49	1.23	1.06	0.00	0.00	0.00	1.11	2.43	1.10	(1.14)	0.02	0.04	0.27
Malic acid	2594.18	2371.89	1663.37	1928.18	1702.22	1647.61	2099.15	2077.36	1785.47	2103.90	1833.82	1781.87	1632.08	1739.73	1659.39	1984.58	1856.97	(0.10)	0.42	0.53	4.42
Maltose	17.23	7.92	13.48	10.70	4.79	4.73	6.12	2.66	2.21	3.45	1.24	1.43	0.72	1.38	2.86	9.81	2.45	(2.00)	0.00	0.01	0.47
Meperidine	0.00	0.22	0.00	0.40	0.44	0.42	0.00	0.47	0.27	0.29	0.35	0.00	0.00	0.25	0.00	0.25	0.18	(0.44)	0.53	0.61	0.12
Methyl galactoside	5.26	4.42	3.32	2.99	2.73	2.67	1.90	2.06	1.69	2.36	2.09	1.82	1.82	2.15	2.10	3.57	2.00	(0.84)	0.00	0.00	0.22
Myo-Inositol	67.73	55.66	61.48	16.36	14.39	11.60	5.14	9.61	4.69	6.50	5.57	4.01	5.71	6.37	5.98	37.87	5.95	(2.67)	0.00	0.01	0.95
Niflumic acid	0.00	1.14	1.02	0.00	0.00	0.00	0.71	1.34	0.19	0.00	0.47	0.00	0.19	0.00	0.00	0.36	0.32	(0.16)	0.89	0.92	0.07
Oxalic acid	71.85	66.80	66.44	129.19	108.93	114.91	63.32	74.12	73.01	75.13	65.83	72.50	72.18	91.65	67.18	93.02	72.77	(0.35)	0.06	0.11	1.75
Palmitic Acid	10.59	7.92	8.87	22.14	12.81	16.58	17.74	9.96	11.72	12.76	11.58	11.98	8.53	10.13	12.06	13.15	11.83	(0.15)	0.53	0.61	0.35
Pentanedioic acid	1.80	1.40	1.43	1.20	0.90	0.00	0.00	1.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.12	0.12	(3.18)	0.00	0.01	0.19
Pentasiloxane	16.09	17.54	13.71	13.14	12.29	12.05	13.71	11.13	11.61	14.23	12.83	13.71	10.59	14.49	12.42	14.14	12.75	(0.15)	0.16	0.24	0.26
Phosphoric acid	74.15	45.08	63.60	56.06	42.03	38.87	34.72	32.06	21.90	20.32	18.38	18.03	14.55	15.43	18.34	53.30	21.52	(1.31)	0.00	0.00	1.16
Pinacol	26.79	19.85	23.11	5.40	5.88	5.51	2.20	4.24	2.34	3.82	3.15	2.47	4.11	4.43	4.15	14.42	3.43	(2.07)	0.01	0.02	0.55
Propanal	1.13	1.19	1.17	0.00	0.33	0.00	0.00	0.17	0.00	0.15	0.00	0.16	0.18	0.00	0.00	0.64	0.07	(3.13)	0.01	0.04	0.12
Propane	129.42	86.12	112.60	23.26	26.66	23.45	8.93	18.78	10.16	15.29	13.94	10.66	18.62	16.91	18.57	66.92	14.65	(2.19)	0.01	0.02	1.21
Propanedioic acid	1995.95	1175.56	1681.84	1022.72	945.90	914.86	512.12	401.78	422.33	462.35	399.81	381.46	405.02	558.61	475.54	1289.47	446.56	(1.53)	0.00	0.00	5.61
Propanoic acid	0.97	0.52	0.35	0.23	0.00	0.00	0.35	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.34	0.06	(2.58)	0.05	0.09	0.11
Ribonic acid	3.00	2.00	2.47	1.74	1.33	1.88	1.75	1.68	1.15	1.59	1.44	1.23	1.21	1.02	1.26	2.07	1.37	(0.60)	0.01	0.02	0.14
Sedoheptulose	16.88	16.70	18.40	16.82	9.41	9.55	15.43	13.19	7.61	17.32	13.74	16.47	16.41	0.00	11.43	14.63	12.40	(0.24)	0.42	0.53	0.18
Shikimic acid	578.15	244.78	325.48	163.80	164.65	246.22	162.64	81.80	93.43	141.68	128.51	136.30	109.35	112.45	145.47	287.18	123.52	(1.22)	0.01	0.02	2.25
Silanol	106.09	87.31	84.97	85.58	67.90	62.43	82.68	69.12	65.34	48.51	47.04	42.90	57.06	66.19	62.63	82.38	60.16	(0.45)	0.01	0.03	0.76
Stearic acid	4.19	2.55	2.64	4.01	2.38	2.01	2.18	1.35	2.33	2.41	1.96	2.17	0.00	0.00	3.04	2.96	1.72	(0.79)	0.04	0.07	0.17
Sucrose	13.28	9.11	12.09	21.68	9.85	12.54	5.18	6.77	5.53	0.00	2.67	0.00	1.95	0.00	0.00	13.09	2.46	(2.41)	0.00	0.00	0.97
Talose	5.67	4.79	2.35	8.65	6.13	6.03	5.28	3.44	3.29	5.49	5.04	4.54	2.97	3.53	3.59	5.60	4.13	(0.44)	0.08	0.13	0.26
Tartaric acid	2.46	1.95	1.77	1.22	1.16	1.21	1.08	0.00	0.86	0.00	0.00	0.00	0.00	0.00	0.00	1.63	0.22	(2.92)	0.00	0.00	0.24
Terephthalic acid	5.85	2.59	2.32	1.89	1.81	1.59	1.96	1.52	1.80	1.36	0.00	1.29	0.00	0.00	0.00	2.68	0.88	(1.60)	0.01	0.04	0.23
Tetrasiloxane	5.66	5.35	6.11	5.54	5.20	7.17	6.37	5.31	6.22	7.40	6.74	8.81	9.26	12.28	11.86	5.84	8.25	0.50	0.04	0.08	0.36

Supplementary Table S1. Cont'd

ethylene	434.32	347.13	432.77	74.59	54.23	45.20	17.64	26.94	14.05	24.84	20.02	14.63	21.05	24.90	20.79	231.37	20.54	(3.49)	0.01	0.02	2.40
carbamate	20.06	18.78	17.15	19.34	18.03	20.32	17.27	16.17	15.96	18.75	17.14	18.90	17.85	19.13	18.52	18.95	17.74	(0.09)	0.08	0.13	0.18
Trisiloxane	4.37	3.83	3.58	3.95	3.54	3.62	3.70	3.56	3.45	4.06	3.28	3.57	3.33	4.61	3.26	3.81	3.65	(0.06)	0.44	0.54	0.10
Xylitol	8.97	6.64	8.11	10.28	6.82	0.00	6.11	4.17	5.25	4.58	4.62	0.00	3.33	0.00	0.00	6.80	3.12	(1.13)	0.03	0.07	0.31
Xylonic acid	8.99	7.68	7.36	6.97	5.85	5.62	5.52	5.51	4.20	4.49	3.81	3.76	2.92	3.33	2.94	7.08	4.05	(0.80)	0.00	0.00	0.34
Xylulose	45.35	41.82	28.17	40.60	36.36	37.58	30.86	24.29	22.52	30.47	27.08	25.22	21.42	25.53	22.10	38.31	25.50	(0.59)	0.00	0.00	0.73
d-Galactose	75.56	157.09	97.02	25.95	21.77	25.82	65.78	57.05	1045.43	31.72	25.20	38.43	26.70	26.98	23.37	67.20	148.96	1.15	0.57	0.65	1.17
d-Mannose	9.62	6.91	5.01	8.24	6.25	5.56	3.52	3.83	2.71	2.25	2.32	2.21	0.97	0.82	0.85	6.93	2.16	(1.68)	0.00	0.00	0.49
propanoic acid	10.41	8.03	6.69	8.55	6.40	5.17	8.79	8.78	6.52	5.12	5.08	4.22	5.68	3.99	4.46	7.54	5.85	(0.37)	0.10	0.17	0.23
trans-2-Hydroxycinnamic acid	2.84	1.19	1.88	1.65	0.80	0.77	1.00	0.55	0.00	0.44	0.38	0.00	0.00	0.00	0.00	1.52	0.26	(2.53)	0.00	0.01	0.20