Study of the Glycosylated Secondary Metabolites in Tea (*Camellia Sinensis* L.) Using UHPLC/Q-TOF/MS

Nontargeted Modification-Specific Metabolomics Approach

Application Note

Food

Authors

Weidong Dai, Junfeng Tan, Dongchao Xie, Pengliang Li, Haipeng Lv, Yin Zhu, and Zhi Lin
Tea Research Institute,
Chinese Academy of Agricultural Sciences,
Hangzhou, China

Meiling Lu
Agilent Technologies (China) Limited

Abstract

Glycosylation is widely involved in a series of biological events in plants and are believed to be beneficial to human health, but studies on this biological process are limited mainly due to the unavailability of a powerful analytical approach to identify the abundant and diverse glycosylated metabolites in plants. This application note describes a nontarget modification-specific metabolomics approach to study glycosylation in tea (*Camellia Sinensis* L.), which was recently reported by Dai, *et al*. [1]. Agilent ultra-high performance liquid chromatography (UHPLC) combined with high-resolution quadrupole time-of-flight mass spectrometry (Q-TOF/MS) was applied to profile the green teas under all-ions in-source fragmentation mode. The acquired data were subjected to a lab-customized workflow to selectively extract glycosylation-related compound features based on the characteristic neutral loss patterns from the specific glycosylation modifications. Further identification of these compounds was based on searching the database directly against the glycosylated metabolites or the corresponding substrates. With this strategy, 202 glycosylated metabolites including glucosylation/galacosylation, rhamnosylation, rutinosylation, and primeverose were detected simultaneously, among which 68 glycosylated metabolites were putatively identified in the green tea infusion. An additional 44 novel glycosylated metabolites were tentatively elucidated based on their MS/MS spectra. This approach allows the user to profile, discover, and identify novel glycosylated metabolites in plant samples.

Agilent Technologies

Introduction

Endogenous modifications of biomolecules are widely observed in living organisms, can modulate a range of biological processes, and have been studied in the field of systems biology extensively [1,2]. These modifications, including glycosylation and acylation, occur not only to large molecules such as genes and proteins, but also to small molecules such as metabolites in plants [3,4]. They can alter the polarity, volatility, chemical stability, and biological activity of metabolites, protecting plants against biotic and abiotic stresses [5,6]. These numerous secondary metabolites contribute significantly to the complexity of the plant metabolome.

Glycosylated secondary metabolites are widely considered better antioxidants, reactive oxygen species scavengers, or coenzymes compared to their unmodified analogs and other primary metabolites [7]. Unfortunately, systematic studies on glycosylation is limited by the unavailability of reliable analytical approaches. With the advancement of ultra-high performance liquid chromatography (UHPLC) and accurate-mass spectrometry with high analytical sensitivity and high resolution, the modified metabolites could possibly be mapped. In particular, the nontargeted modification-specific metabolomics approach demonstrated the potential to simultaneously map the various modifications of metabolites in biological fluid in large scale [8].

Tea is considered a major healthy beverage, and is thought to have some beneficial and protective effects to human health. However, the mechanism is far from clear, due partially to limited knowledge about the endogenous metabolites of tea. It is widely known that tea is rich in catechins, flavones, flavanols, amino acids, and so forth, and these tea metabolites are often observed to be glycosylated, although they have not been studied comprehensively [9,10]. The typical glycosylation observed in tea includes glucosylation, galactosylation, rhamnosylation, rutionosylation, and primeverosylation. Figure 1 lists their corresponding sugar structures. Each of the above glycosylations can add a sugar moiety to the substrate to generate a glycosylated metabolite. During collision-induced dissociation in a tandem mass spectrometer, the glycosylated metabolites are liable to lose the specific sugar moiety as a neutral species. This makes it possible to specifically map these compounds by matching the neutral loss pattern, applying the nontargeted modification-specific metabolomics approach [8]. To study typical tea glycosylation, the detailed workflow for this nontargeted but modification-specific metabolomics approach was described and applied.

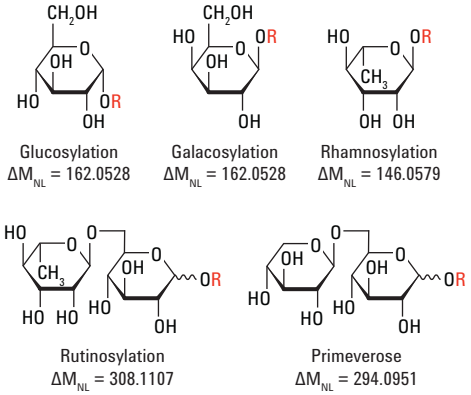

Figure 1. The typical glycosylation modifications in plants. R in each type of glycosylation represents the substrate to be modified; ΔM_{NL} represents the accurate mass difference between the glycosylated metabolites and their substrates due to the neutral loss of their sugar moiety.

Experimental

Chemicals

A group of compounds including kaempferol-3-O-galactoside, kaempferol-3-O-glucoside, quercetin-7-glucopyranoside, L-theanine, D-glucose, quercetin-3-glucoside, 3,5-dicaffeoylquinic acid, chlorogenic acid, epigallocatechin gallate (EGCG), isochlorogenic acid B, theogallin, theaflavin-3-gallate, rutin (quercetin-3-rutinoside), isochlorogenic acid C, and myricitrin (myricetin-3-rhamnoside) were purchased from Sigma (St. Louis, MO, USA) for identity confirmation. Another three compounds, methyl salicylic acid primeveroside, kaempferol 3,7-dirhamnoside, and aesculin, were purchased from ChemFaces (Wuhan, China). In addition, theanine glucoside standard compound was synthesized and purified in the lab, and its structure was determined using ^{1}H-NMR.

Tea sample preparation

Fourteen varieties of tea plant including Jianbohuang, Ningzhou 2, Fuyun 6, Huang Guanyin, Zhenghedabai, Gaoyaqi, Zhuyeqi, Maoxie, Longjing 43, Yuemingxiang, Xicha 5, Fuzao 2, Wannong 95, and Echa 1 were planted in the tea garden of the Tea Research Institute, Chinese Academy of Agricultural Sciences. An equal portion of fresh tea leaves from each variety was collected and mixed thoroughly to obtain as many glycosylated metabolites as possible in the pooled tea leaf sample. The resultant tea leaf samples were subjected to a green tea manufacturing process following the previously reported procedure [11]. In addition, the fresh tea leaves from each variety of tea plant were made into green tea separately using the same procedure. The resultant green tea sample was ground into powder, and 30 mg of it was thoroughly mixed with 1.5 mL of 60 % methanol solution (v/v) by vortexing for 20 seconds. The mixture was then ultrasonicated for 10 minutes followed by centrifugation at 10,000 g for 10 minutes. The collected supernatants with the major tea metabolome were passed through a 0.22 µm filter for LC/Q-TOF/MS analysis. Figure 2 shows a schematic of the procedure.

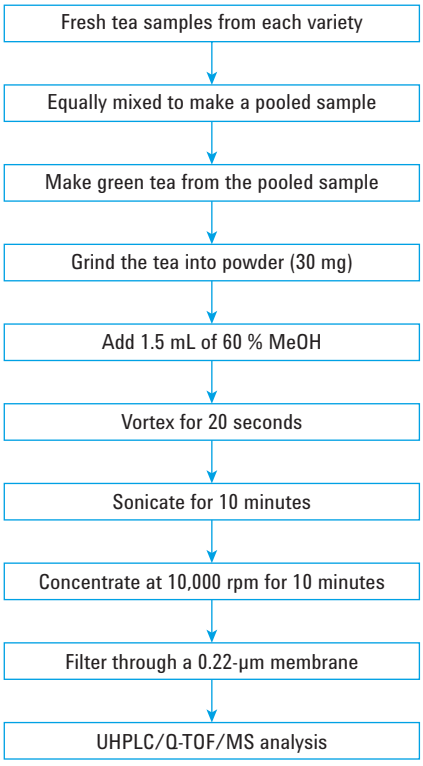

Figure 2. Procedure for preparation of the tea sample.

LC/Q-TOF/MS Analysis of tea samples

The prepared tea extract was subjected to analysis using an Agilent 1290 Infinity II UHPLC combined with high-resolution quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF/MS) under gradient elution and positive ionization mode with slight modification from previous work [12]. Table 1 specifies the detailed experimental conditions for UHPLC and Q-TOF/MS.

Detailed LC/MS conditions

Table 1. Instrument Conditions

LC conditions	
Instrument	Agilent 1290 Infinity II LC System with built-in degasser
Autosampler	Agilent 1290 Infinity II Autosampler with temperature control
Column temperature	Agilent 1290 Infinity II Thermostatted Column Compartment
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm
Column temperature	40 °C
Mobile phase	A) Aqueous solution containing 5 mmol/L ammonium acetate and 0.1 % formic acid B) Methanol containing 5 mmol/L ammonium acetate and 0.1 % formic acid
Flow rate	0.40 mL/min
Injection volume	1.0 µL
Post time	4 minutes
Gradient elution profile	0–4 minutes: 10–15 %B 4–7 minutes: 15–25 %B 7–9 minutes: 25–32 %B 9–16 minutes: 32–40 %B 16–22 minutes: 40–55 %B 22–28 minutes: 55–95 % 28–30 minutes: 95 %
ESI-MS/MS conditions	
Instrument	Agilent 6540 Q-TOF LC/MS system with Agilent dual Jet Stream electrospray ionization source
Ionization mode	Positive
Drying gas temperature	300 °C
Drying gas flow rate	8 L/min
Nebulizer gas pressure	35 psi
Sheath gas temperature	300 °C
Sheath gas flow rate	11 L/min
Capillary voltage	3,500 V
Nozzle voltage	500 V
Scanning mode	TOF scan and target MS/MS scan
Scan range	Scan range: 100–1,000 (MS)/50–1,000 (MS2)
Collision energy	0, 5, 10, 15, 20, 25, 30, 35, 40,45 eV for all ions ionization (presudo in source ionization)
Reference ions	121.0509, 922.0098

Workflow for nontargeted modification-specific metabolomics analysis

To conduct nontargeted modification-specific metabolomics analysis, the scanning TOF spectra were acquired in all-ions in-source fragmentation mode with CE ranging from 0 to 45 eV, providing the basis for follow-up studies.

The scanning TOF/MS data acquired at different collision energies for the pooled tea sample were initially subjected to feature extraction using Agilent MassHunter Qualitative Analysis software (Version 7.0, Agilent Technologies, Santa Clara, CA). The results were then imported into Agilent Mass Profiler Professional (MPP) software (Version 13.1, Agilent Technologies, Santa Clara, CA) for peak alignment. The resultant peak table was exported and subjected to a lab-customized software, Neutral Loss MSFinder, for neutral loss matches [8]. The ions with the same coelution profiles and their mass differences consistent with the characteristic neutral losses of *m/z* 162.0528, 146.0579, 308.1107, and 294.0951 (Figure 1) corresponded to the precursor ions of the glycosylated metabolites and substrate ions as the fragments dissociated from the precursors with glucosylation/galactosylation, rhamnosylation, rutinosylation, and primeverosylation modifications, respectively. The error tolerances of retention time (t_R) and mass for neutral loss (ΔM_{NL}) matches were set as Δt_R <0.1 minutes, and ΔM_{NL} <0.002 Da respectively. The resultant glycosylated metabolites or the substrates were identified against the customized tea PCDL, Metlin, and human metabolome database (HMDB) databases. Those not found in the databases were elucidated based on their MS/MS spectra with the aid of Agilent MassHunter MSC software (v.7.0). Some of the tentatively identified compounds were further confirmed using authentic standards in the lab. Figure 3 shows the schematic diagram for the developed workflow.

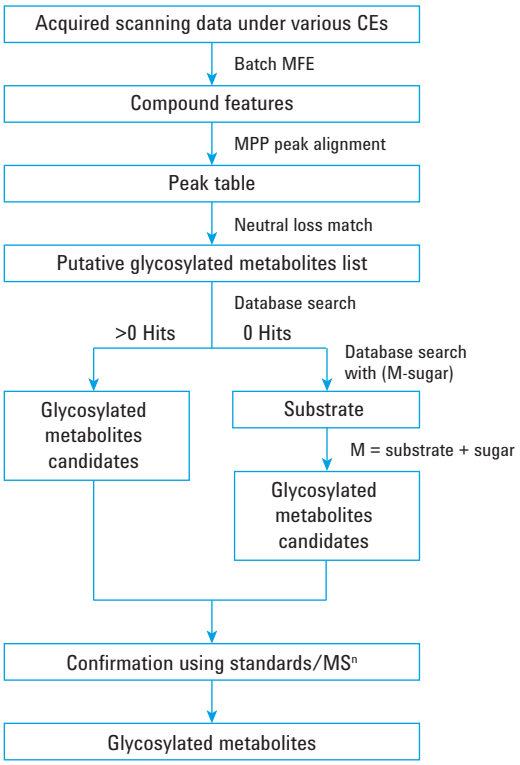

Figure 3. Schematic illustration of the detailed workflow for the nontarget modification-specific tea glycosylation analysis. MFE = molecular feature extraction; M represents the glycosylated metabolites; substrate is the compound covalently bonded to one or several sugar moieties to form glycosylated metabolites; the n in the MS^n can be 2 or 3, and when n = 3 it means that the Q-TOF was operated under ion source fragmentation followed by further dissociation in the collision cell before accurate TOF scanning of the third-stage fragments.

Differentiation of tea varieties based on their glycosylation patterns

Traditionally, tea manufacturers select specific tea plant types to make different types of tea such as green, black, woolong, yellow, and puer teas. This demonstrates that each type of tea plant has its own suitability for manufacturing one specific type of tea. To take a deep look at whether the differences in glycosylation patterns among the 14 tea varieties are correlated with the suitability of tea making, principle component analysis (PCA) based on the relative abundance of the identified glycosylated compounds was conducted.

Results and Discussion

Strategy and workflow for identification of metabolite glycosylation

Figure 1 shows that the endogenous glycosylation adds a certain sugar moiety such as a monosaccharide or disaccharide to the metabolite substrates through covalent binding. When exerting appropriate energy to the glycosylated metabolites in the gas phase, the added mono- or disaccharide ligands can easily be dissociated from the precursors through the neutral loss pathway. The mass difference between the glycosylated metabolites and their substrates corresponds to the mass of the sugar moiety, and can act as a characteristic mass indicator for each specific glycosylation type. To simultaneously acquire as many glycosylation features of a complex sample as possible, nonselective all-ions in-source collision-induced dissociation (ISCID) was applied.

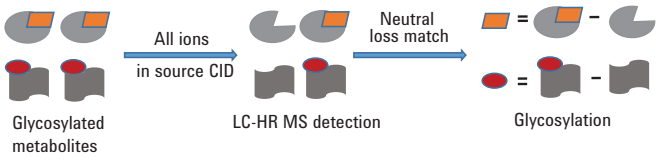

Figure 4. *The strategy for mapping the glycosylation patterns in tea. The sugar moiety (neutral loss) is determined by matching the intact glycosylated metabolite precursor ion and the fragment ion produced by ISCID. The orange parallelogram and the dark red oval represent two different types of sugar moiety, respectively, and the grey shapes represent the substrates to be glycosylated.*

In the instrument configuration, the quadrupole of the Q-TOF/MS was set to pass all ions from the ionization source to the collision cell in which collision energy was exerted. The resulting fragments were further transferred to the TOF analyzer for accurate mass scanning. To selectively retrieve the ion features related to glycosylation, a strategy was developed based on the specific neutral loss match [8]. Only the pair of ion species with mass difference corresponding to one specific sugar moiety neutral loss (Figure 4) and with the same chromatographic coelution profiles would signify one type of glycosylation. Figure 3 shows a further detailed workflow, to identify the glycosylated metabolites directly, or to identify their substrates, and gradually reconstruct the precursors through the neutral loss pattern.

As a proof-of-principle test, quercetin-3-glucoside, kaempferol-3-O-galactoside, myricetin-3-rhamnoside, quercetin-3-rutinoside, and methyl salicylic acid primeveroside were initially examined as the model compounds for each type of common glycosylation. Theoretically, dissociation of a sugar moiety from the compounds produced from glucosylation, galactosylation, rhamnosylation, rutinosylation, and primeverosylation would generate MS spectra containing the typical neutral mass loss of 162.0528 ($C_6H_{10}O_5$), 146.0579 ($C_6H_{10}O_4$), 308.1107 ($C_{12}H_{20}O_9$), and 294.0951 ($C_{11}H_{18}O_9$) respectively, when suitable collision energy is applied. High-resolution of Q-TOF/MS allows the user to distinguish glucosidation/galactosylation ($m/z_{(NL)}$ = 162.0528) from caffeoylation ($m/z_{(NL)}$ = 162.0317), and rhamnosylation ($m/z_{(NL)}$ = 146.0579) from coumaroylation ($m/z_{(NL)}$ = 146.0368), respectively, which are not distinguishable when using conventional triple quadrupole or Q-trap-based tandem mass spectrometry. For example, in the ISCID MS/MS spectrum of kemopferol-3-galacoside, the *m/z* of 287.0553 corresponds to the [M-sugar] ion species, which is 162.0529 lower than the precursor ion of kemopferol-3-galacoside (449.1082) (Figure 5A). Quercetin-3-glucoside and myricetin-3-rhamnoside were found with neutral losses of 162.0520 and 146.0578, corresponding to glucosidation (Figure 5B) and rhamnosylation (Figure 5C), respectively. Similar features were also observed for two other model compounds (data not shown). The spectra for model compounds demonstrate that combining neutral loss features with chromatographic coelution characteristics makes it possible to retrieve glycosylation patterns selectively from a complex sample such as tea.

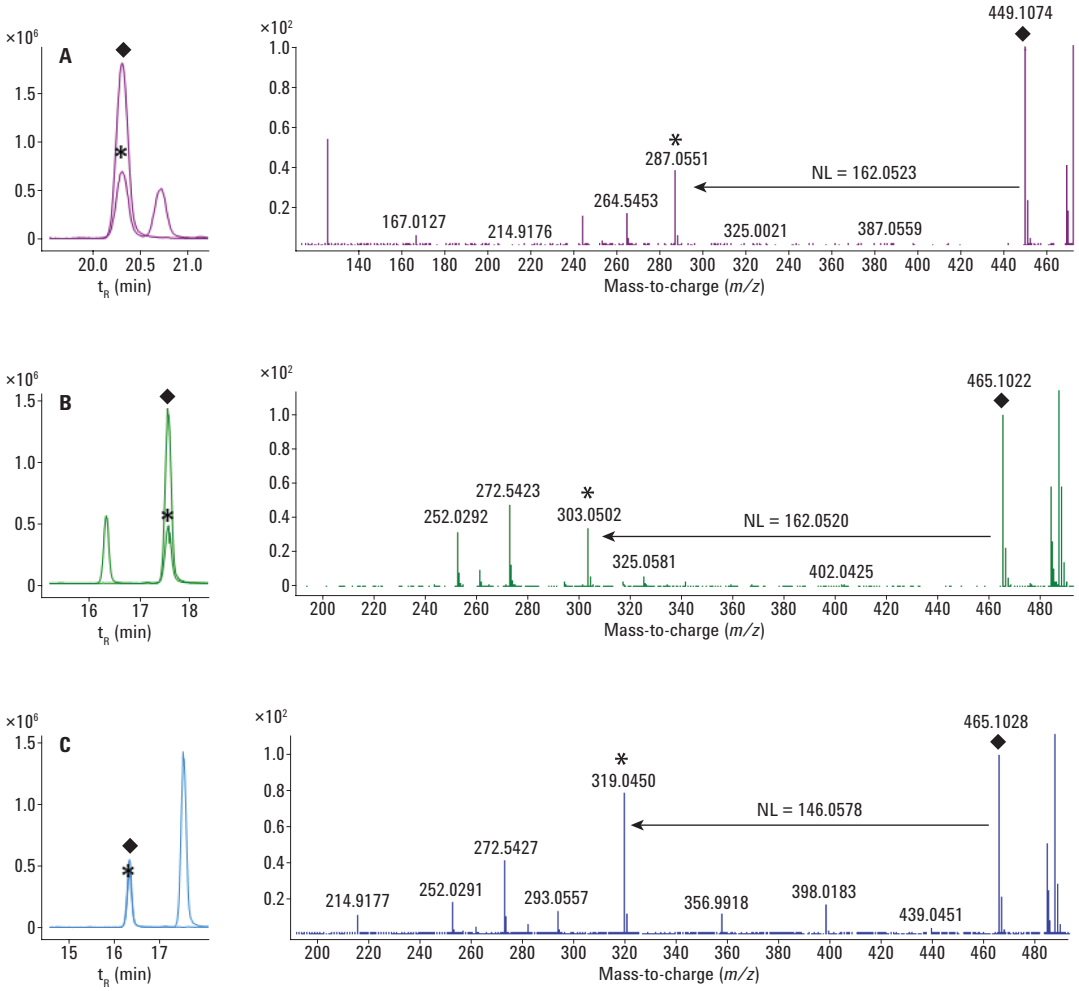

*Figure 5. Typical overlay of the extracted ion chromatograms for precursor and substrate ions of the glycosylated compounds (left) and its ISCID-based fragmentation pattern (right). A) Kaempferol galactoside; B) Quercetin-3-glucoside; C) Myricetin 3-rhamnoside. The experimentally determined neutral losses of m/z 162.0523, 162.0520, and 146.0578 correspond to the losses of galactose, glucose, and rhamnose, respectively. The ♦ represents the precursor ion, and the * represents the substrate ion (the fragment derived from neutral loss of the sugar moiety).*

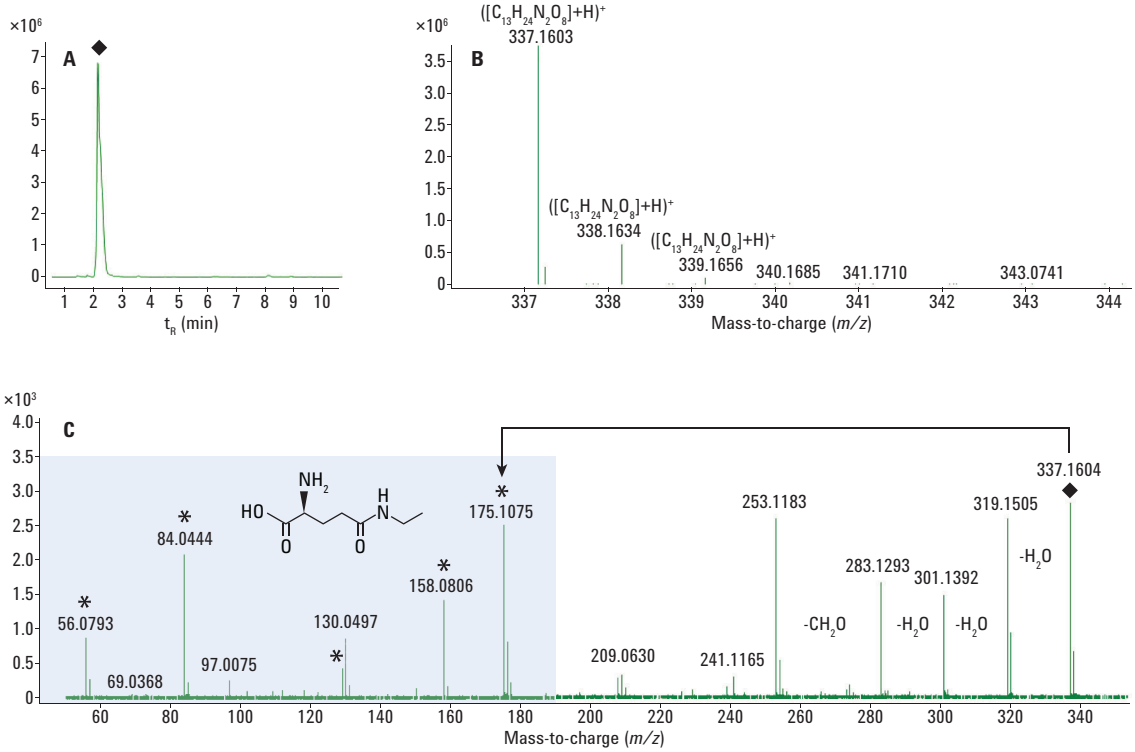

Figure 6. Identification of one novel compound with m/z at 337.1603 and retention time at 2.26 minutes. A) Extracted ion chromatogram; B) Accurate TOF/MS spectrum with calculated formula; C) Target MS/MS of the compound. ***Note:*** *there is a peak at 175.1075 corresponding to a neutral loss of 162.0529 (glu/gal) from the precursor ion of 337.1604 in trace C, and there are also characteristic MS/MS peaks with * labeled in the shaded region, suggesting that the compound contains a theanine substrate.*

Application of the developed strategy to identify glycosylated metabolites in the green tea

Using the workflow shown in Figure 3, the acquired scanning TOF/MS data under varying collision energies were processed. After searching the neutral loss matches, a total of 202 compounds were found to be glycosylated metabolites in a pooled sample, 120 were glucosylated/galactosylated metabolites, 38 were rhamnosylated metabolites, 21 were rutinosylated metabolites, and 23 were primeverosylated metabolites. Further target MS/MS analyses of these compounds demonstrated that 144 out of the 202 compounds can generate neutral losses deriving from the specific glycosyl moieties (Table 2), and most of the remaining 58 compounds could not generate qualified MS/MS spectra due to low abundance.

Optimizing database search strategy to enhance identification coverage

The high analytical sensitivity and high resolution of LC/Q-TOF/MS facilitate acquiring accurate TOF/MS spectra for thousands of compounds simultaneously from one single plant sample such as green tea infusion. However, identification of these metabolites is very challenging and has been the bottleneck in plant metabolomics studies since only a very small portion of chromatography and MS signals can be structurally interpreted or documented in a database [13,14]. After searching the available databases including the customized tea PCDL, Metlin, and HMDB with tens of thousands of entries, only 68 out of 144 CID-MS^2 validated glycosylated compounds were further structurally interpreted.

Table 2. Selected Glycosylated Metabolites with Structural Elucidations in Green Tea

Assigned compound	*m/z* (ESI+)	t_R (min)	Adduct	Mass error (ppm)	Glycosylation type	MS^2	Included in database
3′-Methoxyfukiic acid rhamnoside	455.1153	1.65	M+Na	-1.5	Rha	148, 184, 130, 258, 309	No
Glucosylcellotriose	705.184	1.65	M+K	-1.4	Glu/Gal	611, 543, 432	Yes
Nicotinic acid glucoside	286.0913	1.66	M+H	-2.9	Glu/Gal	124, 252	No
Serine rhamnoside	252.1065	1.67	M+H	-5.0	Rha	148, 134 118, 106	No
L-Glutamic acid diglucoside	472.1658	1.67	M+H	-0.6	Glu/Gal	134, 148, 292, 310	No
Methylguanine triglucoside	652.23	1.68	M+H	-1.2	Glu/Gal	148, 325, 335, 490	No
Glutamic acid glucoside	310.114	1.68	M+H	2.4	Glu/Gal	114, 130, 148, 226, 246	No
Guanine triglucoside	638.2163	1.68	M+H	1.8	Glu/Gal	325, 134, 145, 163, 476, 541	No
Aesculin	341.0865	7.89	M+H	-0.6	Glu/Gal	139, 151, 165, 123, 179	Yes
1-O-Caffeoylglucose	343.1032	8.21	M+H	2.5	Glu/Gal	297, 139, 307, 181	Yes
1-(4-Hydroxyphenyl)-1,2,3-propanetriol 2-O-*beta*-D-glucopyranoside	347.1306	8.61	M+H	-8.8	Glu/Gal	301, 285, 185, 203	Yes
Salicylic acid *beta*-D-glucoside	323.0731	8.81	M+Na	-2.0	Glu/Gal	185, 161, 203, 153	Yes
(1S,2S,4R,8S)-p-Menthane-1,2,8,9-tetrol 2-glucoside rhamnoside	535.2352	10.09	M+Na	-1.7	Rha	407, 357, 365, 389	No
Methyl 3,4-dihydroxy-5-prenylbenzoate 3-glucoside	399.1644	10.18	M+H	-1.4	Glu/Gal	163, 131, 237, 279	Yes
Pantothenic acid primeveroside	514.2137	10.26	M+H	1.3	Pri	382, 393, 273, 291, 220	No
Lusitanicoside	465.1723	10.29	M+Na	-1.8	Glu/Gal	303, 277, 175	Yes
Myricetin 3-glucoside	481.0970	14.41	M+H	-1.4	Glu/Gal	319	Yes
Myricetin rutinoside	627.1546	14.95	M+H	-1.5	Rut	175, 319, 455	No
Apigenin digulucoside	595.1651	15.27	M+H	-1.1	Glu/Gal	433, 313, 271, 415	No
Kaempferol 3,7-dirhamnoside	579.1699	15.42	M+H	-1.6	Rha	433, 303, 313, 415	Yes
Quercetin 3-O-galactosylrutinoside	773.2128	16.01	M+H	-0.9	Rut	303, 465, 611	Yes
Quercetin 3-galactoside	465.1018	16.21	M+H	-2.0	Glu/Gal	303	Yes
Quercetin-3-O-galactoside	465.1019	16.91	M+H	-1.8	Glu/Gal	303	Yes
Aeglin	533.1986	17.04	M+Na	-1.4	Glu/Gal	481, 499, 469, 409, 371	Yes
Rutin	611.1597	17.27	M+H	-1.6	Rut	303, 465, 561	Yes
Isoquercitrin	465.1020	17.29	M+H	-1.6	Glu/Gal	303	Yes
Kaempferol 7-glucosylrutinoside	757.2176	17.95	M+H	-1.2	Rut	287, 449, 595, 611	Yes
Cinnamic acid diglucoside	473.1659	18.84	M+H	1.2	Glu/Gal	371, 127, 321, 311	Yes
Benzyl 2,6-dihydroxybenzoate 2-glucoside	429.1149	18.97	M+Na	-1.6	Glu/Gal	249, 267, 287,321	Yes
Astragalin	449.1070	19.01	M+H	-1.9	Glu/Gal	287, 85	Yes
Methoxybrassinin rutinoside	575.1727	19.01	M+H	-0.1	Rut	331, 398, 267, 429	No
Kaempferol 3-glucosylrutinoside	757.2175	19.02	M+H	-1.4	Rut	287, 449, 595	Yes
Kaempferol-3-O-galactoside	449.1070	19.21	M+H	-1.9	Glu/Gal	287	Yes
Linalyl oxide rutinoside	479.2482	19.46	M+H	-1.0	Rut	252, 295, 259, 333, 171	No
Ginkgolide C rutinoside	749.2492	24.77	M+H	-0.9	Rut	147, 309, 441, 587	No
Moracin C primeveroside	605.2249	24.79	M+H	3.3	Pri	457, 441, 221, 311, 385	No

Glu: glucosidation; Gal: galactosylation; Rha: rhamnosylation; Rut: rutinosylation; Pri: primeverosidation. Fragment ions highlighted in blue represent the substrate ion of the glycosylated metabolite. Please refer to the supporting information in Reference 1 when the complete compound list is required.

(Table 2).

To enhance the coverage for identification of the glycosylated metabolites, the substrate ([M-sugar]$^+$) was subjected to database search identification when there was no hit for the queried ion features in the putative glycosylated metabolites list (Figure 3). The tentative structure of the queried ion feature could be reconstructed by combining the substrate part and the sugar moiety part. Using this strategy, an additional 44 glycosylated metabolites in the pooled green tea infusion, which were considered novel compounds, were further elucidated. Among them, one typical ion feature (m/z = 337.1599, t_R = 2.26 minutes) showed an NL of 162.0524, which corresponded to a glycosylated metabolite (Figure 6). This ion feature did not match with any candidate compounds in the databases, but the substrate ion feature m/z = 175.1071 matched with theanine, a characteristic free amino acid of the tea, in the database. Hence, the ion feature m/z 337.1599 was tentatively identified as theanine glucoside, which was not reported previously. The identification of this novel compound was further confirmed using a synthesized theanine glucoside standard in the lab.

Variations of the glycosylated metabolites among green teas from different varieties

To examine the variation of glycosylation among different varieties of tea, the tea samples obtained from 14 varieties were subjected to the nontargeted metabolomics analysis. With PCA based on the identified glycosylated metabolites, the pooled samples (QC samples in blue) of 14 green teas were crowded together in the center of the PCA score plot (Figure 7), suggesting very good data reproducibility and reliability during the metabolomics analysis. In addition, the tea samples can easily be separated into two parts in the score plot. On the right side of PCA score plot are Wannong 95, Zhuyeqi, Gaoyaqi, Jianbohuang, Fuyun 6, and Ningzhou 2, which displayed similar glycosylation patterns; those on the left side are Longjing 43, Xicha 5, Echa 1, Zhenghedabai,

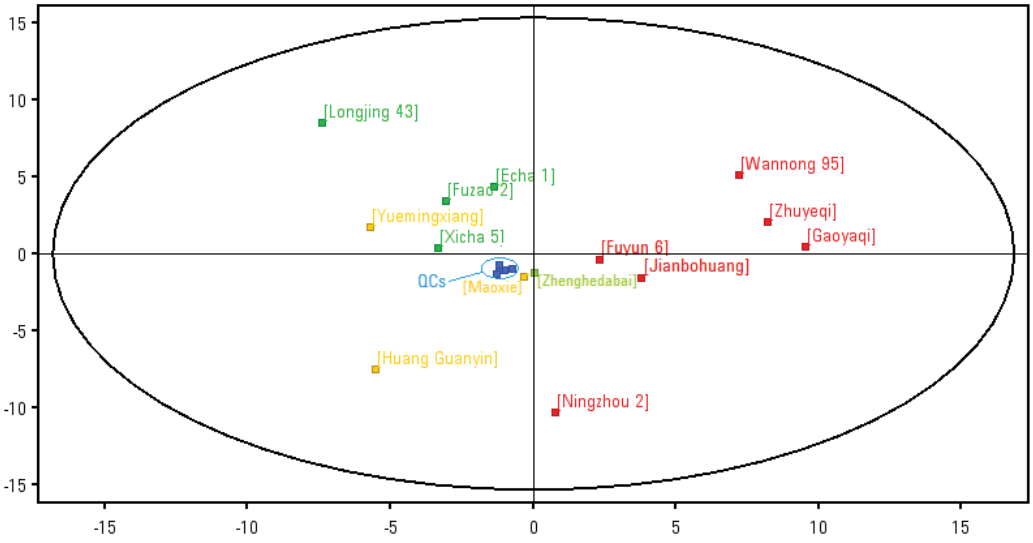

Figure 7. Score plot for principal component analysis of green tea samples from 14 varieties. The varieties in red are typically suitable for making fully fermented tea such as black tea; the varieties in green and orange are typically suitable for making green tea and semifermented tea such as wooloog, respectively.

Fuzao 2, Maoxie, Yuemingxiang, and Huangguanyin. They also have similar glycosylated metabolites profiles.

Some of the glycosylated metabolites contributed significantly to the variations for the tea samples on the left and right parts of the PCA plot. Galactocylated compounds showed elevated levels in the tea on the left of the PCA score plot, whereas glucosylation shows the opposite tendency. For example, kaempferol-3-O-galactoside and quercetin-3-O-galactoside displayed significantly higher levels ($p < 0.05$) in the left part; kaempferol-3-O-rutinoside and kaempferol 3-glucosylrutinoside showed the reverse tendency ($p < 0.05$) (Figure 8). Empirically, the tea plants on the right are suitable for making black tea, and those on the left are normally used for making green tea and semifermented tea.

Hence, the ratio of galactosylation/glucosylation in tea plants may be an important factor for making different types of tea.

Conclusion

In this study, a UHPLC/Q-TOF/MS-based nontargeted modification-specific metabolomics approach is detailed and successfully applied to profile and identify the secondary metabolites with glucosylation, galactosylation, rhamnosylation, rutinosylation, and primeverosylation in the green tea infusion. This described workflow greatly enhances the identification coverage of glycosylated metabolites, and improves the capability for structural elucidation of unknown

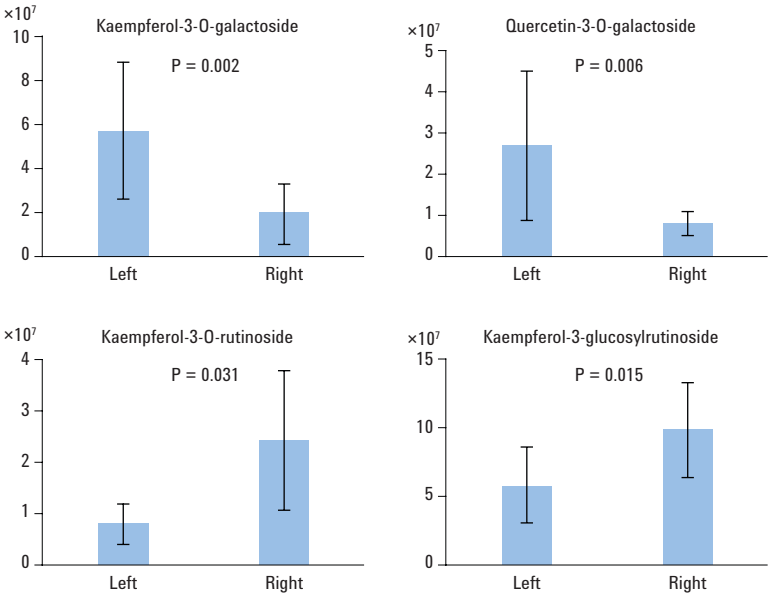

Figure 8. The typical distinctive glycosylated metabolites between the varieties (Zhenghedabai, Fuzao 2, Maoxie, Longjing 43, Yuemingxiang, Xicha 5, Huang Guanyin, and Echa 1) located in the left part of the PCA score plot (Figure 6), and the varieties (Jianbohuang, Ningzhou 2, Gaoyaqi, Zhuyeqi, Wannong 95, and Fuyun 6) located in the right part of the PCA score plot.

metabolites. It can further be extended to profile many other significant modifications in plants.

References

1. W. D. Dai, *et al.* "Nontargeted modification-specific metabolomics investigation of glycosylated secondary metabolites in tea (Camellia sinensis L.) based on liquid chromatography-high resolution mass spectrometry" *J. Agric. Food Chem*. **64**, 6783-6790 (2016**)**.
2. D. O. Croci, *et al.* "Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors" *Cell* **156**, 744-758 (2014).
3. J. Cheng, *et al.* "Unraveling the mechanism underlying the glycosylation and methylation of anthocyanins in peach" *Plant Physiology* **166**, 1044-1058 (2014).
4. L. Cui, *et al.* "Identification of UDP-glycosyltransferases involved in the biosynthesis of astringent taste compounds in tea (Camellia sinensis)" *J. Exp. Bot.* **67**, 2285-2297 (2016).
5. T. Huan, *et al.* "MyCompoundID MS/MS Search: Metabolite Identification Using a Library of Predicted Fragment-Ion-Spectra of 383,830 Possible Human Metabolites" *Anal. Chem*. **87**, 10619-10626 (2015).
6. L. Li, *et al.* "MyCompoundID: using an evidence-based metabolome library for metabolite identification" *Anal. Chem*. **85**, 3401-3408 (2013).
7. V. Arbona, *et al.* "Metabolomics as a Tool to Investigate Abiotic Stress Tolerance in Plants" *Intern. J. Mol. Sci.* **14**, 4885-4911 (2013).
8. W. Dai, *et al.* "Nontargeted Modification-Specific Metabolomics Study Based on Liquid Chromatography–High-Resolution Mass Spectrometry" *Anal. Chem*. **86**, 9146-9153 (2014).
9. A. Drewnowski, C. Gomez-Carneros. "Bitter taste, phytonutrients, and the consumer: a review" *Am. J. Clin. Nutr*. **72**, 1424-1435 (2000).
10. H.P. Lv, *et al.* "Bioactive compounds from Pu-erh tea with therapy for hyperlipidaemia" *J. Functional Foods* **19**, 194-203 (2015).
11. W. Dai, *et al.* "Nontargeted analysis using ultraperformance liquid chromatography–quadrupole time-of-flight mass spectrometry uncovers the effects of harvest season on the metabolites and taste quality of tea (*Camellia sinensis* L.)" *J. Agric. Food Chem.* **63**, 9869-9878 (2015).
12. J. Tan *et al.* "Study of the dynamic changes in the non-volatile chemical constituents of black tea during fermentation processing by a non-targeted metabolomics approach" *Food Res. Intern.* **79**, 106-113 (2016).
13. J. Chen, *et al.* "Practical approach for the identification and isomer elucidation of biomarkers detected in a metabonomic study for the discovery of individuals at risk for diabetes by integrating the chromatographic and mass spectrometric information" *Anal. Chem*. **80**, 1280-1289 (2008).
14. J. Mitchell, *et al.* "Development and in silico Evaluation of Large-Scale Metabolite Identification Methods Using Functional Group Detection for Metabolomics" *The FASEB J.* **29**, 567.522 (2015).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
July 13, 2017
5991-8066EN

Agilent Technologies