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Foodomics Platform for the Assay of Thiols in Wines with Fluorescence Derivatization and Ultra Performance Liquid Chromatography Mass Spectrometry Using Multivariate Statistical Analysis

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ABSTRACT: The presence of specific volatile and aminothiols in wine is associated with quality, worth, price, and taste. The identification of specific thiol-containing compounds in various wines has been reported in many valuable and interesting works. In this study, a novel foodomics assay of thiol-containing compounds, such as free aminothiols and related conjugates, was developed using ultra performance liquid chromatography (UPLC) with fluorescence (FL) and electrospray (ESI) time-of-flight mass spectrometric (TOF/MS) detections. FL specific derivatization was applied along with multivariate statistical analysis. First, the optimal experimental conditions were studied using representative thiols, such as L-cysteine, *N*-acetyl-L-cysteine, cysteamine, and L-glutathione, and then the UPLC-FL derivatization and separation steps were fixed for the subsequent screening of unknown thiol-containing compounds. The screening assay consisted of monitoring the UPLC-TOF/MS peaks of unknown thiols, which decreased due to the derivatization as compared to the nonderivatized thiols. The principal component analysis of the UPLC-TOF/MS data could be well-differentiated and categorized into two groups. The orthogonal signal correction partial least-squares discriminant analysis, the so-called S-plot, showed that the quality differentiation is directly related to the decrease of native thiols and increase of derivatized thiols. With this strategy, the mass difference from the derivatization reagent ($+m/z$ 198) could be utilized for the identification of these thiols using the FL peaks retention time and metabolomics-databases. The presence of L-glutathione in rice wine was for the first time reported on the basis of the available metabolomics-databases and standard matching. This novel concept based on foodomics could be applied in food analysis for the ready screening of specific functional compounds by exploiting the various derivatization modes available.

KEYWORDS: *foodomics, wine, thiols, UPLC, multivariate statistical analysis*

INTRODUCTION

Thiol-containing compounds have been reported to be very important for the various aroma impacts of wine. Although the presence of a thiol-containing compound is associated with the quality, worth, price, and taste in wine, it has also been documented for the various kinds of brands, lots, and earmark wines. Volatile thiols, in particular, 3-mercaptohexan-1-ol (3-MH), have been found to be an important aroma of Sauvignon blanc.[1] Although the presence of 3-MH is commonly associated with Sauvignon blanc, it has also been documented in various kinds of wines.[2−4] Moreover, other volatile thiols, such as 4-mercapto-4-methylpentan-2-one and 3-mercaptohexyl acetate, have been identified as key molecules of young wines elaborated by the production.[5,6] Aminothiols, which are formed during the fermentation process by yeast, have been identified as precursors of the previously cited volatile thiols.[7] Thus, thiol-containing compounds have been the focus of importance regarding the aroma for individual wines with local characters and appraisal factors.[8]

Japanese sake is a popular and traditional wine made from steamed rice by multiple fermentations. For the evaluation of this rice wine, the screening profiles of various metabolites and sugar compounds were analyzed by liquid chromatography with mass spectrometry (LC/MS) for pasteurized and unpasteurized rice wine during storage.[9] Amino acids, which are important for the quality of premium rice wine, have been analyzed by Fourier transform near-infrared spectroscopy.[10] Many other organic acids and peptides are included in rice wines.[11,12] Thus, a wide range of molecules would be needed to be simultaneously studied, analyzed, and profiled for the evaluation of quality and value in various statuses.[13] On the other hand, a few studies have reported thiol-containing compounds in rice wine.[14,15]

The screening assays of various thiols in wine have been discussed in many studies.[16] Several derivatization reagents for the stabilization and LC or gas chromatography (GC) detection of thiols have been described for the screening of

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specific thiol-containing compounds in foods using a multiple classification analysis.[17] However, it is impossible to screen unknown thiols from a multipeak chromatogram using these techniques. Numerous analytical methods for the assay of particular thiols in biological samples were developed using LC with ultraviolet (UV), fluorescence (FL), and MS detections along with various derivatizations.[18−21] We have previously reported that a specific thiol-derivatization reagent, 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F), can be advantageously used for the determination of thiols in biological samples.[22−25] The present work aimed to show a novel foodomics LC approach with multivariate statistical analysis such as principal component analysis (PCA) and orthogonal signal correction partial least-squares discriminant analysis (OSC-PLS-DA) for the discovery of thiol-containing compounds in rice wine using SBD-F derivatization (Figure 1).

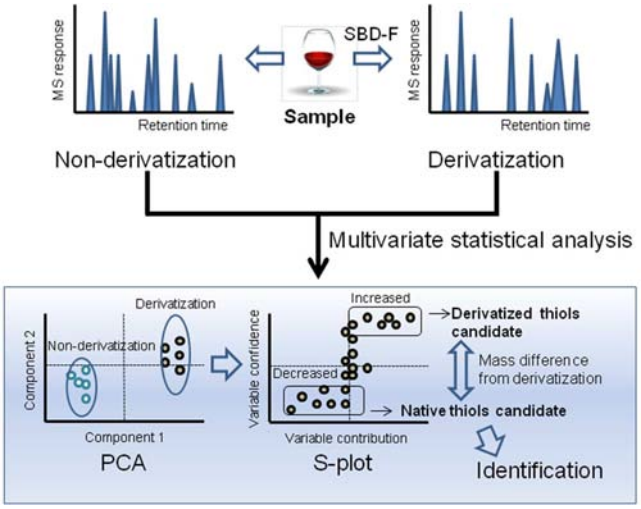

Figure 1. Proposed foodomics platform for the novel screening assay of thiol-containing compounds in wine by UPLC-FL/TOF/MS with derivatization and multivariate statistical analysis.

In the LC screening assay, the MS peaks of unknown thiol-containing compounds in wine decreased after SBD-F derivatization as compared to nonderivatized thiols. Thus, if the presence of several thiols is detected in a sample, the PCA of the UPLC-TOF/MS data with derivatization and non-derivatization could well-differentiate and categorize two groups. OSC-PLS-DA, the so-called S-plot, showed that the quality of differentiation is explained by at least the decreased (native thiols) and increased (derivatized thiols) components. Using these data, the mass difference from the SBD-F reagent could be utilized for identification of these thiols from the FL peaks retention time and the metabolomics-databases. This foodomics approach could be a useful screening discovery method of unknown thiol-containing compounds in wine and food samples.

MATERIALS AND METHODS

Reagents. L-Cysteine (CYS), *N*-acetyl-L-cysteine (ACYS), cysteamine (CA), and reduced L-glutathione (GSH) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Sodium tetraborate was purchased from Wako Pure Chemical Co. (Osaka, Japan). The 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) and disodium ethylenediamine tetraacetate, dehydrate (EDTA) were obtained from Dojindo Chemical Co. (Kumamoto, Japan). Trifluoroacetic acid (TFA) and acetonitrile (for HPLC) were obtained from Kanto Pure Chemical Co. (Tokyo, Japan). Tri-*n*-butylphosphine (TBP) was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). The purified water was obtained from a Milli-Q purifying system (Millipore Co., Bedford, MA). Wine samples were obtained from a local store in Shizuoka, Japan. Pure standard solution (1.0 mg/mL) was prepared with 5 mM EDTA in water. Mixed standard solutions were prepared by diluting an aliquot of the standard in 5 mM EDTA.

UPLC Analysis. The UPLC system was a Waters Acquity H Class (Waters Co., Milford, MA). The reversed phase analysis was performed using an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) at 40 °C. The injection volume was 5 μL. The mobile phase consisting of solvent A, 0.1% TFA in water, and solvent B, 0.1% TFA in acetonitrile, was delivered at the flow rate of 0.2 mL/min. Two gradient modes of this mobile phase were used for the simple separation of the standard solution/free thiols (type A) and absolute separation of the various free thiols from the conjugates with TBP in the samples (type B). The gradient elution of type A was as follows: 0.0 min [A/B: 99/1], 30 min [A/B: 2/98], and 30.1 min [A/B: 99/1]. The gradient elution of type B was as follows: 0.0 to 5.0 min [A/B: 99/1], 35 min [A/B: 2/98], and 35.1 min [A/B: 99/1]. The elution of the derivatized thiols was monitored at 380 nm excitation and 510 nm emission wavelengths.

MS Analysis. The separated compounds were detected by a Waters LCT Premier XE time-of-flight mass spectrometer (TOF/MS) (Waters Co., Milford, MA). The electrospray (ESI) (positive ionization mode) conditions were as follows: capillary voltage was 3.0 kV, sample cone was 15 V, source temperature of 120 °C, and desolvation temperature of 350 °C. The cone and desolvation gas flows were 50 and 650 L/h, respectively, and were obtained using a nitrogen source. The analytical mode and dynamic range were the V mode and normal. The aperture 1 voltage was 15 V. For calibration, the reference solution used 4 μg/mL leucine enkephalin (m/z 556.28, <2 ppm) in 0.1% formic acid in water/acetonitrile (50/50, v/v). The scan mode was used from m/z 100 to 1000.

Derivatization Using SBD-F. The sample or standard solutions were adjusted to 100 μL and transferred to plastic test tubes. To this solution were then added 100 μL of 10 mM SBD-F in water and 100 μL of 0.1 M tetraborate buffer (pH 9.2) at 60 °C for 20 min. After incubation, as soon as possible, this solution was automatically injected into the UPLC system. These solutions were useful for the solubility, stability, and UPLC studies. No degradation was observed for the analytes and the SBD-F derivatized species during the experimental period.

Decomposition of Oxidized Form Corresponding Thiols by TBP. Rice wine or white wine sample (100 μL) was added to 30 μL of 10% TBP in acetonitrile at 4 °C for 30 min. These conditions were based on a previous work reported elsewhere.[26] This sample solution was then derivatized using the above protocol.

Multivariate Statistical Analysis for the Derivatization. The UPLC-TOF/MS data were analyzed for peak detection and alignment from m/z 100 to 1000, and exported for PCA and OSC-PLS-DA by MarkerLynx XS V4.1 SCN803 (Waters Co., Milford, MA). The method parameters were as follows: mass tolerance = 0.05 Da, Apex Track Peak Parameters, peak width at 5% height (s) = 15/peak-to-peak baseline noise = 50, apply smoothing = yes, collection parameters, intensity threshold (counts) = 100/mass window = 0.05/retention time window = 0.10, noise elimination level = 6, deisotope data = yes.

Identification of Native Thiol-Containing Compounds by Databases. The elemental compositions of the single thiol-containing compounds on the S-plot were identified on the basis of the accurate mass and retention time, and the values of mDa (the difference from the exact mass) and i-FIT (the correctness of isotope patterns of elemental composition; the lower i-FIT normalized values mean high) of each candidate were shown. Three compounds with the good values of the mDa and i-FIT level were extracted from these prospective formulas, and matching the MS spectra of the unknowns to standard model compounds. The MarkerLynx XS V4.1 SCN803 combined lists of biomarkers candidates were extracted from five metabolomics databases: NIST (http://www.nist.gov/pml/data/asd.cfm), MassBank

(http://www.massbank.jp/), KEGG (http://www.kegg.com/), BioCyc (http://biocyc.org/), and Food and Agriculture Organization of Unitied Nations (http://faostat.fao.org/site/291/default.aspx). A mass tolerance of 5.0 mDa was set as well as a maximum elemental composition of C = 500, H = 1000, N = 200, O = 200, S = 10, P = 10, and Cl = 10. The software automatically filters compounds from different libraries that have the same KEGG.[27,28]

RESULTS AND DISCUSSION

Screening Assay Using UPLC-FL. A mixture of representative thiols, such as CYS (10 μg/mL), ACYS (10 μg/mL), CA (1 μg/mL), and GSH (10 μg/mL), was used for developing the screening assay. CYS, ACYS, and GSH are present in various wines and are involved in the specific reaction of wine flavors.[29,30] CA is a very famous compound, being a useful antioxidant found in vegetables such as grapes.[31] Thus, these compounds were used for the development of the screening assay of thiols in rice wine samples. First, the reaction time and UPLC separation of these derivatized compounds were investigated. The derivatization reaction time was found to be completed in 20 min, and therefore this reaction time was selected (Figure 2). The UPLC chromatograms for the blank [A], standard [B], rice wine without derivatization [C], and rice wine with derivatization [D] are reported in Figure 3. Several reversed-phase columns and mobile phase (added formic acid, acetic acid, and TFA: concentration of 0.1%) were evaluated for the separation of the derivatized thiols (Figure 3B). In these results, a good separation of CYS (retention time (RT): 2.6 min), CA (RT: 3.5 min), GSH (RT: 7.0 min), and ACYS (RT: 7.4 min) could be achieved using the UPLC BEH C18 column with a mobile phase consisting of 0.1% TFA in water/acetonitrile. The monitoring emission and excitation wavelengths (3D mode) were selected at 380 and 510 nm, respectively. The recovery of these standards in UPLC-FD chromatogram in rice wine was found to be 95−98% (n = 3). Thus, in this study, rice wine samples would be targeted for the determination of unknown thiols using UPLC-FL. On the other hand, the blank data (Figure 3A and C) indicated that no other background peaks were detected. In addition, the 10% ethanol in pure water was analyzed using same condition, and indicated that no other background peaks were detected. On

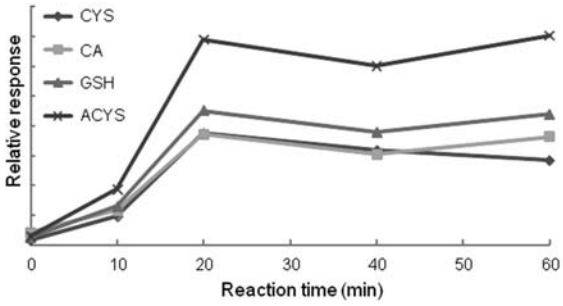

Figure 2. Investigation of optimal conditions for the derivatization of thiols. Standard solution was added to 100 μL of 10 mM SBD-F in water, and 100 μL of 0.1 M tetraborate buffer (pH 9.2) at 60 °C for optimal 20 min.

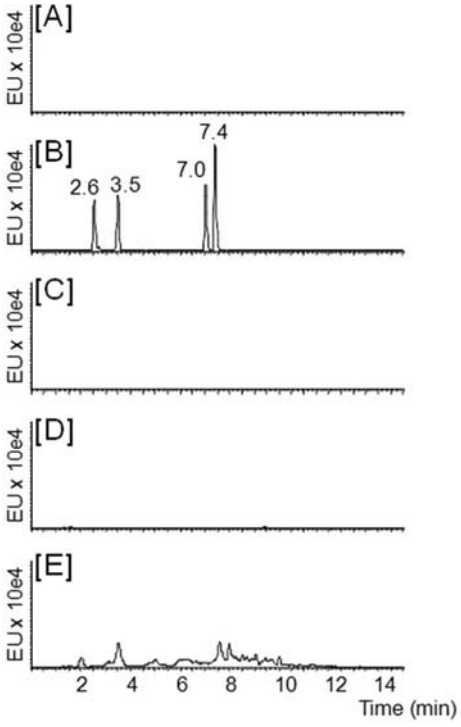

Figure 3. UPLC-FL chromatograms of thiols under optimal conditions with derivatization and TBP. [A] Blank solution (pure water) was added to 10 mM SBD-F in 0.1 M tetraborate buffer (pH 9.2) at 60 °C for 20 min. [B] Standard solution (CYS [2.6 min], CA [3.5 min], GSH [7.0 min], and ACYS [7.4 min]) was added as in [A]. [C] Sample solution (rice wine) was added to 0.1 M tetraborate buffer (pH 9.2) at 60 °C for 20 min. [D] Sample solution (rice wine) was added as in [A]. [E] Sample solution (rice wine) was added to 10 mM SBD-F with 10% TBP in 0.1 M tetraborate buffer (pH 9.2) at 60 °C for 20 min.

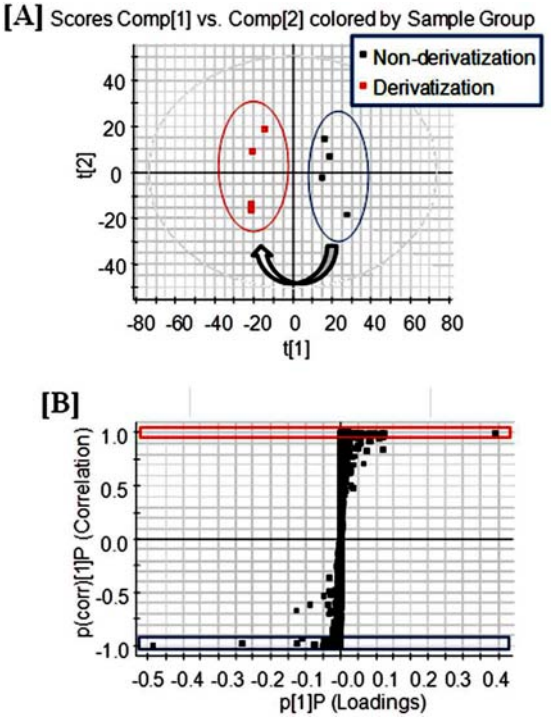

Figure 4. Multivariate statistical analysis of UPLC-TOF/MS with derivatization of rice wine. [A] PCA of UPLC-TOF/MS data with non- and derivatized conditions (n = 5) showing well-differentiated and categorized groups for the same rice wine treated with TBP. [B] S-plot showing that the increased (red box) and decreased (blue box) peaks after derivatization can be the specific derivatized unknown thiol-containing compounds in rice wine.

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Table 1. MS Data and Prospective Formulas from Rice Wine by UPLC-ESI/TOF/MS with FL Derivatization

no.	native thiols (m/z)	derivatized thiols (m/z)	prospective formulas
1	133.03	331.20	
2	150.06	348.17	$C_5H_{11}NO_2S$
3	159.02	357.23	$C_5H_7N_2O_2S$, $C_5H_8N_2PS$
4	170.14	368.16	
5	173.14	371.16	$C_{10}B_{21}S$
6	196.06	394.27	$C_8H_{10}N_3OS$, $C_6Hi_5NO_2PS$
7	201.07	399.04	$C_8H_{13}N_2O_2S$, $C_6H_{18}O_3PS$, $C_{13}H_{13}S$
8	204.09	402.21	$C_{12}H_{14}NS$, $C_6H_{14}N_5OS$
9	212.19	410.30	
10	233.06	431.03	$C_9H_9N_6S$, $C_{13}H_{13}O_2S$, $C_8H_{13}N_2O_4S$
11	244.16	442.24	$C_5H_{22}N_7O_2S$, $C_{10}H_{22}N_5S$
12	251.07	449.08	
13	269.10	467.07	$C_8H_{13}N_8OS$, $C_{12}H_{17}N_2O_3S$, $C_nH_{18}N_4PS$
14	276.10	474.08	
15	278.12	476.09	$C_{11}H_{16}N_7S$, $C_9H_{21}N_5OPS$, $C_{10}H_{20}N_3O_4S$
16	285.10	483.07	$C_{11}H_{18}N_4OPS$, $C_{10}H_{21}O_7S$, $C_{11}H_{17}N_4O_3$
17	298.19	496.19	$C_{13}H_{34}NPS$, $C_{16}H_{28}NO_2S$, $C_{10}H_{28}N_5O_3S$
18	308.10	506.06	**$C_{10}H_{17}N_3O_6S$ (GSH)**
19	330.07	528.05	
20	334.12	532.09	$C_8H_{26}N_5O_3P_2S$, $C_{10}H_{21}N_7O_2PS$, $C_9H_{25}N_3O_6PS$
21	353.25	551.35	$C_{17}H_{24}N_5S$
22	354.14	552.23	$C_{18}H_{20}N_5OS$, $C_{17}H_{24}NO_5S$, $C_{12}H_{21}N_9PS$
23	383.14	581.11	
24	396.18	594.22	
25	406.15	604.23	
26	419.20	617.31	$C_{10}H_{33}N_{10}O_2P_2S$
27	421.33	619.33	$C_{23}H_{50}O_2PS$, $C_{25}H_{45}N_{20}S$, $C_{19}H_{46}N_6PS$
28	439.17	637.16	
29	498.21	696.18	$C_{29}H_2O_2S$, $C_{26}H_{33}P_2S$, $C_{25}H_{23}N_6S$
30	522.24	720.21	$C_{18}H_{37}N_9O_5PS$, $C_{19}H_{33}N_{13}OPS$, $C_{20}H_{32}NnO_4S$
31	555.19	753.19	
32	583.25	781.22	

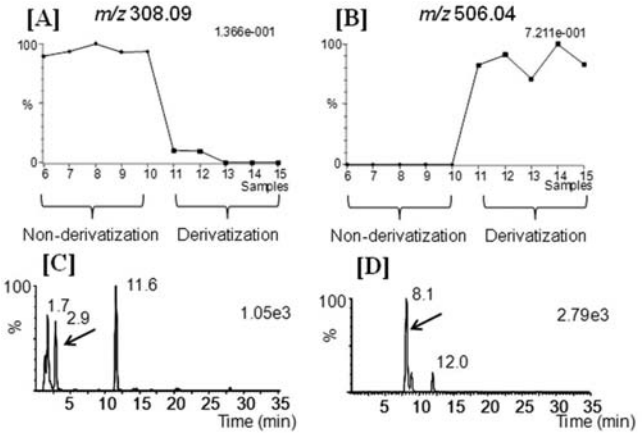

Figure 5. Ideal pattern of GSH for the rice wine sample. [A] Peak response pattern of m/z 308 (native GSH peak). [B] Peak response pattern of m/z 506 (derivatized GSH peak). [C] Extracted single ion monitoring UPLC-ESI/TOF/MS chromatogram of m/z 308 in the case of nonderivatization for native GSH (retention time, 2.9 min). [D] Extracted single ion monitoring UPLC-ESI/TOF/MS chromatogram of m/z 506 in the case of derivatization for derivatized GSH (retention time, 8.1 min).

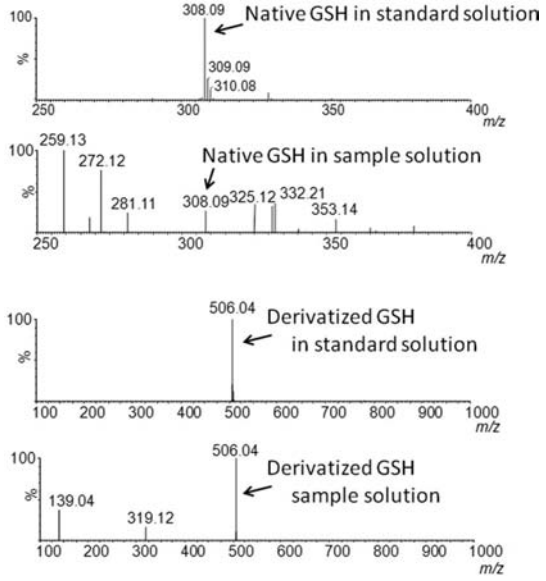

Figure 6. MS spectra of GSH for the identification of unknown thiols in rice wine using our novel approach.

the other hand, no peaks of free thiols were detected in the rice wine samples using this system (Figure 3D). Volatile thiols are present in white wines samples at levels of around 100 ng/L.[32] Thus, the rice wine samples were found to have the same pattern as grape wine for the presence of free thiols at very low concentration levels. On the other hand, Figure 3E showed that several distinct peaks were detected in the rice wine due to the cleavage of the soluble conjugates to their corresponding thiols by TBP. Thus, the UPLC-FL chromatogram indicated that unknown thiol-containing compounds would be present in the rice wine samples from decomposition of oxidized form corresponding thiols by TBP. This UPLC-FL system could then be directly connected to the TOF/MS detector for the identification of unknown thiols in wine sample.

Screening Assay Using UPLC-TOF/MS with Derivatization. The metabolic profile using LC−MS and a multivariate statistical analysis represents an emerging and powerful discipline that provides a useful picture of biomaps through the study of potential metabolites for the discovery of new biomarkers and patterns in metabolomics study. Commonly, one system such as TOF/MS was used for the identification and screening of unknown compounds in metabolomics study. Recently, it is believed that the integration of LC−MS with derivatization could add value to enhance the capabilities of LC−MS-based analytical platform and could provide an alternative strategy to tackle some difficult analytical problems.[33] Some of the advantages of this integration include increased analyte stability during preparation and analysis, increased retention time of polar compounds using reverse-phase columns, enhanced analyte ionization efficiency in an MS interface, increased analyte molecular weight for improving MS selectivity, and enabled protein/peptide analysis with a stable isotope-labeling and/or fluorescent-labeling reagent specific to a certain functional group.[33] In our foodomics platform, the UPLC-FL detection of unknown derivatized peaks (retention

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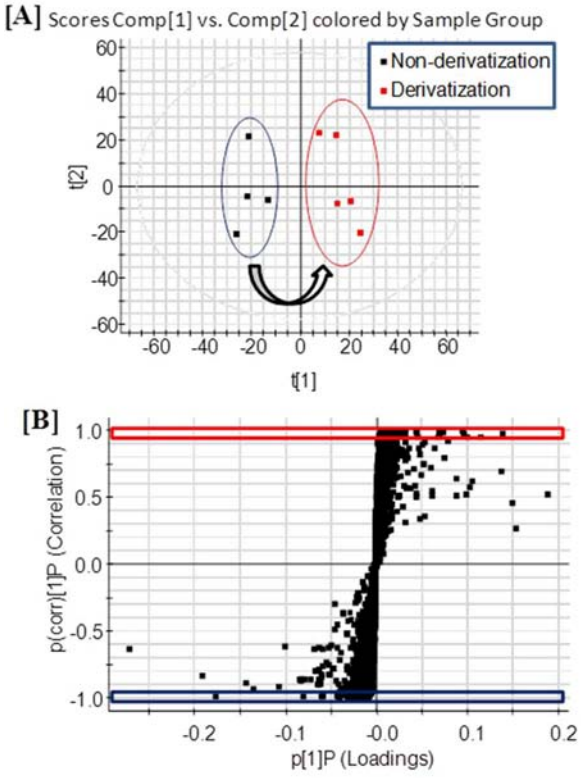

Figure 7. Multivariate statistical analysis of UPLC-TOF/MS with derivatization in white wine (Beauchatel Sauvignon Blanc from France). [A] PCA of UPLC-TOF/MS data with non- and derivatized conditions (n = 5) showing well-differentiated and categorized groups for the same white wine treated with TBP. [B] S-plot shows that the increased (red box) and decreased (blue box) peaks after derivatization can be the specific derivatized unknown thiol-containing compounds in white wine with TBP.

time) was used for the advantages of UPLC integration system. In fact, it is impossible to pick specific derivatized peaks and process these m/z values related to the derivatization of thiols for the identification of unknown compounds based on UPLC-TOF/MS data, multivariate statistical analysis, and database research. Therefore, we arranged the present metabolic profiling analysis exploiting the derivatization of specific thiols in rice wine and proposed this novel approach. Figure 1 shows that same sample, nonderivatized and derivatized, provides two MS scan chromatograms, which can be compared by multivariate statistical analysis. Many peaks of unknown compounds and the background were detected using the ESI-positive mode for the multivariate statistical analysis. Thus, the m/z values of the background from the derivatization condition are eliminated such as m/z 163.13, 203.19, 219.19 (from derivatization reagent), 235.19, 250.97, 284.33, 291.20, 305.16, 349.18, 393.21, 399.25, 437.24, 481.26, 500.35, and 601.34 using the condition of 10 mM SBD-F in water and 0.1 M tetraborate buffer (pH 9.2) with or without TBP.

Multivariate Statistical Analysis of UPLC-TOF/MS Data with Derivatization. LC−MS followed by a multivariate statistical analysis has been applied to the metabolic profiling. Different types of samples for PCA and OSC-PLS-DA models were compared for the class separation between the positive and control samples.[34,35] Using these derivatized components, the mass difference from the SBD-F reagent could not be utilized for the PCA and OSC-PLS-DA based on the original

Table 2. MS Data and Prospective Formulas from White Wine by UPLC-ESI/TOF/MS with FL Derivatization

no.	native thiols (m/z)	derivatized thiols (m/z)	prospective formulas
1	183.08	381.22	$C_5H_{15}N_2O_3S$
2	203.12	401.17	$C_9H_{19}N_2OS$
3	211.12	409.25	$C_6H_{19}N_4OS$, $C_6H_{20}N_4PS$, $C_{12}H_{19}OS$
4	213.12	411.28	$C_7H_{21}N_2O_3S$, $C_7H_{22}N_2OPS$
5	229.21	427.27	
6	241.16	439.12	$C_5H_{21}N_8OS$, $C_8H_{26}N_4PS$, $C_9H_{25}N_{203}S$
7	267.17	465.32	$C_{11}H_{27}NO_3S$, $C_8H_{26}N_4PS$, $C_{10}H_{28}N_4PS$
8	269.16	467.34	$C_{12}H_{31}P_2S$, $C_9H_{25}N_4O_3S$, $C_{15}H_{25}O_2S$
9	270.19	468.38	$C_{15}H_{28}NOS$
10	272.17	470.11	$C_{14}H_{26}NO_2S$, $C_9H_{28}N_5O_2S$
11	273.23	471.30	$C_{15}H_{33}N_2S$
12	274.21	472.33	
13	279.12	477.11	
14	289.05	487.31	$C_{11}H_9N_6O_2S$
15	293.23	491.26	$C_{14}H_{33}N_2O_2S$, $C_{19}H_{33}S$
16	298.19	496.38	$C_{13}H_{34}NP_2S$, $C_{10}H_{28}N_5O_3S$, $C_{16}H_{28}NO_2S$
17	308.09	506.06	**$C_{10}H_{17}N_3O_6S$ (GSH)**
18	313.18	511.46	$C_{17}H_{29}O_3S$, $C_{16}H_{30}N_2PS$, $C_{13}H_{25}N_6OS$
19	315.20	513.23	$C_{12}H_{32}N_4OSCl$
20	322.19	520.28	$C_{12}H_{24}N_{11}OS$
21	343.19	541.34	$C_{19}H_{27}H_4S$, $C_{18}H_{31}O_4S$, $C_{11}H_{33}N_6P_2S$
22	373.36	571.43	$C_{22}H_{49}N_2S$
23	415.24	613.45	
24	429.25	627.30	
25	457.32	655.38	$C_{17}H_{46}N_8O_2PS$, $C_{21}H_{50}N_2O_4PS$, $C_{22}H_{49}O_7S$
26	460.29	658.52	
27	479.31	677.53	
28	558.22	756.44	
29	605.38	803.36	$C_{21}H_{53}N_{10}O_8S$, $C_{24}H_{59}N_6O_5P_2S$
30	617.42	815.49	$C_{27}H_{61}N_4O_9S$, $C_{26}H_{62}N_6O_{65}PS$, $C_{30}H_{66}O_8PS$
31	629.46	827.56	$C_{34}H_{65}N_2O_6S$, $C_{32}H_{70}O_7PS$, $C_{33}H_{66}N_4O_3PS$
32	696.18	894.36	$C_{52}H_{26}NS$, $C_{46}H_{26}N_5OS$, $C_{45}H_{30}N0_5S$
33	757.58	955.65	$C_{38}H_{89}N_4P_4S$, $C_{31}H_{79}N_{14}OP_2S$, $C_{36}H_{82}N_6O_6PS$
34	774.38	972.49	
35	793.46	991.44	$C_{34}H_{73}N_{10}OP_4S$, $C_{29}H_{58}N_{22}OPS$, $C_{33}H_{77}N_6O_5P_4S$

data from scanned chromatograms. It is impossible to use the m/z values from the scan chromatogram in the derivatization for the multivariate statistical analysis due to the many peaks such as the nonderivatized components. In this study, we propose a novel approach of the multivariate statistical analysis for the discovery of thiol-containing compounds in wine samples using the SBD-F derivatization. The MS peaks from the scan chromatograms with non- and derivatized sample were extracted for the multivariate statistical analysis. The PCA and S-plot for the rice wine sample are shown in Figure 4. In this result, the PCA of the UPLC-TOF/MS data with TBP for the non- and derivatized rice wine sample could be well-differentiated and categorized into two groups of one rice

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wine with TBP (Figure 4A). Thus, the presence of thiol-containing compounds in the sample showed a differentiated PCA pattern. In the S-plot, the increased (red box) and decreased (blue box) peaks after derivatization can be the specific derivatized unknown thiols-containing compounds in the rice wine (Figure 4B). In addition, one set of increased and decreased components was indicated on the basis of the difference m/z value of 198 for the single thiol-reaction. The S-plot showed that the quality differentiation is explained by at least the decreased (native thiols) and increased (derivatized thiols) components based on differentiation of m/z 198 with a correlation of 0.9. A total of 419 peaks were focused on to identify possible thiol candidates such as 32 compounds based on FL peak retention times. Table 1 shows a list of unknown single thiol-containing compounds based on various databases. These molecular formula candidates and m/z values were indicated for future works to identify thiols in a rice wine sample. We showed that an example of this is in the discovery of GSH in rice wine, which identified our ideal pattern such as extracted MS chromatograms and peak response pattern (Figure 5). As an example, Figure 6 shows the positive ESI spectra of the GSH standard and samples for non- and derivatized conditions. We first discovered the presence of GSH in rice wine using our novel approach technique. This first screening assay would be useful for the identification of other unknown thiols in various wine samples.

Application to the Wine. Recently, Roland et al. reported a review of various thiols in wine.[16] They showed a useful list regarding to the reported thiol-containing compounds in various wine samples based on references, name, and odors of thiols. However, no MS information regarding the identification was shown in any of their results. In this study, we can use TOF/MS data regarding the comparison between future reports for the identification of thiols in white wine. On the basis of white wine (Beauchatel Sauvignon Blanc 2011 from France), the PCA and S-plot from the UPLC-TOF/MS with non- and derivatized conditions are shown in Figure 7. The S-plot showed that the quality differentiation is explained by the native and derivatized thiol-containing compounds based on differentiation of m/z 198 with a correlation of 0.9. A total of 665 peaks were focused on the need to identify possible thiol candidates such as 35 compounds based on FL peak retention times (Table 2). We indicated the exploratory presence of thiol-containing compounds in white wine using our concept of foodomics. This first screening assay would be used to identify other unknown thiol-containing compounds in various wine samples.

Conclusions. An original UPLC-FL/TOF/MS assay for thiol-containing compounds based on a multivariate statistical analysis is described. Wine samples can be readily derivatized for the screening of their free thiols content and direct analysis using the UPLC system. In our study, the oxidized and reduced forms of thiols were targeted due to the cleavage of the soluble conjugates to their corresponding thiols by TBP. In this result, an unknown thiol-containing compound, such GSH, was successfully detected in a rice wine sample. However, the identification of all of the thiol-containing compounds would require the use of various reference databases, matching standards, and future studies to correct the native mass spectra during the MS process for foodomics. Among them, we can advocate a new concept of derivatization using the PCA and S-plot of the UPLC-TOF/MS data for the screening assay of unknown thiol-containing compounds. This concept based on foodomics could be applied to use the easy screening assay of specific functional compounds by exploiting the various derivatization modes described in the literature for the discovery of essential functions in foods.[34−38]

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ABBREVIATIONS USED

3-MH, 3-mercaptohexan-1-ol; LC/MS, liquid chromatography with mass spectrometry; UV, ultraviolet detection; FL, fluorescence detection; SBD-F, 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate; UPLC, ultra performance liquid chromatography; ESI, electrospray ionization; TOF-MS, time-of-flight mass spectrometer; CYS, L-cysteine; ACYS, *N*-acetyl-L-cysteine; CA, cysteamine; GSH, reduced L-glutathione; EDTA, disodium ethylenediamine tetraacetate, dehydrate; TFA, trifluoroacetic acid; TBP, tri-*n*-butylphosphine

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