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Comparative Analysis of Phytochemicals and Polar Metabolites from Colored Sweet Potato (*Ipomoea batatas* L.) Tubers

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Abstract We determined the phytochemical diversity, including carotenoids, flavonoids, anthocyanins, and phenolic acids, in sweet potatoes (*Ipomoea batatas* L.) with distinctive flesh colors (white, orange, and purple) and identified hydrophilic primary metabolites. Carotenoid content was considerably higher in orange-fleshed sweet potatoes, wherein β-carotene was the most plentiful, and anthocyanins were detected only in purple-fleshed sweet potatoes. The levels of phenolic acids and flavonoids were relatively higher in purple-fleshed sweet potatoes than those in the other two varieties. Forty-one primary and 18 secondary metabolite profiles were subjected to multivariate statistical analyses, which fully distinguished among the varieties and separated orange- and purple-fleshed sweet potatoes from white-fleshed sweet potatoes based on the high levels of sugars, sugar alcohols, and secondary metabolites. This is the first study to determine comprehensive metabolic differences among different color-fleshed sweet potatoes and provides useful information for genetic manipulation of sweet potatoes to influence primary and secondary metabolism.

Keywords: carotenoid, metabolomics, partial least squares discriminant analysis, phenolic acid, sweet potato

Introduction

Phytochemicals with high free-radical-scavenging activity may play important roles in reducing the risk of heart disease, common cancers, and other degenerative diseases (1,2). Therefore, it is important to consume a diet high in antioxidants to reduce the harmful effects of oxidative stress. Sweet potatoes (*Ipomoea batatas* L.) are one of the most important food crops worldwide, and their leaves, stems, and tubers are being consumed by an increasing number of people, particularly in Asian countries (3). This crop provides rich sources of starch, dietary fiber, minerals, vitamins, and phytochemicals with antioxidant activities, such as carotenoids, flavonoids, and other phenolic compounds. Sweet potatoes have numerous health benefits, such as anti-mutagenic, anti-diabetic, and hepato- and cardio-protective effects, attributable to their phytochemical content (4). Despite the importance of sweet potatoes, few large-scale systematic studies have been conducted on their genomics, transcriptomics, proteomics, or metabolomics (5).

Research to identify valuable compounds present in these plants is necessary for the food and breeding industries, as considerable attention has been paid to edible plants rich in bioactive compounds with antioxidant activities and other beneficial physicochemical properties. Qualitative variations in the nutrients and phytochemical profiles of sweet potatoes could contribute to differences in health-promoting properties. Therefore, we determined the variability in bioactive secondary metabolites and identified the core primary metabolites in sweet potatoes with different-colored tubers. The primary metabolite profile is closely related to phenotype and important nutritional characteristics (6). In this study, hydrophilic primary metabolites from sweet potatoes were profiled using gas chromatography-time-of-flight mass spectrometry (GC-TOFMS) to determine phenotypic variations and relationships among metabolite contents. A GC-TOFMS-based metabolic profiling analysis facilitates rapid and highly sensitive detection of plant metabolites from the central pathways of primary metabolism (7,8). Carotenoids, flavonoids, anthocyanins, and phenolic acids were quantified as bioactive secondary metabolites to evaluate the quality of three sweet potato varieties. The data were subjected to multivariate statistical analyses, partial least-squares discriminant analysis (PLS-DA), Pearson's correlation analysis, and hierarchical clustering analysis (HCA). These data will be

useful to assist future sweet potato breeding strategies or develop new functional foods with increased health benefits.

Materials and Methods

Samples and chemicals Seeds of sweet potatoes (*Ipomoea batatas* L.) cv. 'Yulmi' (white), 'Juhwangmi' (orange), and 'Sinjami' (purple) were provided from National Institute of Crop Science in Rural Development Administration (Suwon, Korea) and stored at 4°C. Three sweet potato cultivar seeds were germinated in a greenhouse and seedlings were transferred to the experimental farm at Bioenergy Crop Research institute (Muan, Korea; 58'10.89''N, 126°27'22.33''E) on May 20, 2013. After 120 days, the sweet potatoes were harvested (Fig. 1) on September 17, 2013 and stored for 90 days in the storeroom which consistently maintained 95% humidity and 18°C degree. For component analysis, three biological replicates of each cultivar were then freeze-dried at −80°C for at least 72 h and ground into a fine powder using a planetary mono mill (Pulverisette 6; Fritsch, Idar-Oberstein, Germany). All chemicals used in this study were analytical grade. Methanol and chloroform, used as extraction solvents, were purchased from J.T. Baker (Phillipsburg, NJ, USA). Lutein, zeaxanthin, 13*Z*-β-carotene, β-carotene, (all-*E*)-β-carotene, 9*Z*-β-carotene were obtained from CaroteNature (Lupsingem, Switzerland). Kaempferol, luteolin, myricetin, quercetin, ribitol, β-Apo-8'-carotenal,sinapic acid, vanillic acid,and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Cyanidin and peonidin were purchased from Extrasynthese (Genay, France). Methoxyamine hydrochloride was purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Syringic, 3,4,5-trimethoxy-cinnamic, *p*-hydroxybenzoic, ferulic, and acetic acids were acquired from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *p*-Coumaric acid was obtained from MP Biomedicals (Solon, OH, USA). Pyridine and *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluroacetamide (MTBSTFA) with 1% *tert*-butyldimethylchlorosilane (TBDMCS) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Extraction and analysis of carotenoids Carotenoids were extracted according to the method described previously (9). β-Apo-8'-carotenal was added as an internal standard (IS). The extracts from the sweet potatoes were dried under a stream of nitrogen and dissolved in 50:50 (v/v) dichloromethane/methanol before high-performance liquid chromatography (HPLC) analysis. The carotenoids were then separated in a C30 YMC column (250×4.6 mm, 3 μm; YMC Co., Kyoto, Japan) by an Agilent 1100 HPLC instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a photodiode array detector. Chromatograms were generated at 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate; solvent B consisted of 100% methyl *tert*-butyl ether. Gradient elution was performed at 1 mL/min under the following conditions: 0 min, 90% A/10% B; 20 min, 83% A/17% B; 29 min, 75% A/25% B; 35 min, 30% A/70% B; 40 min, 30% A/70% B; 42 min, 25% A/75% B; 45 min, 90% A/10% B; and 55 min, 90% A/10% B. Calibration curves were drawn for quantification by plotting four concentrations of the carotenoid standards according to the peak area ratios with IS.

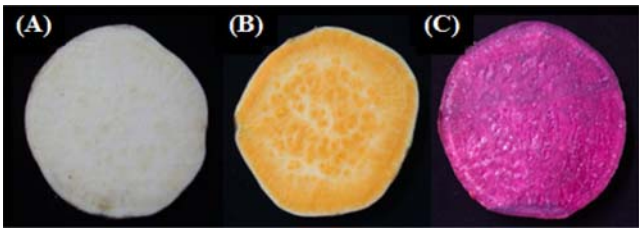

Fig. 1. Images of the three sweet potato varieties (*Ipomoea batatas* L.). (A) cv. Yulmi, (B) cv. Juhwangmi, (C) cv. Sinjami.

Extraction and analysis of flavonoids Extraction and measurement of flavonoid aglycones by HPLC were performed as described by Kim *et al*. (10). Flavonoids from the sweet potatoes (0.1 g) by adding 1.2 mL of 50% MeOH containing 1.2 M HCl at 80°C in a water bath for 2 h. The crude suspensions were centrifuged at 10,000×*g* at 4°C for 5 min. The extracts were passed through 0.22 μm Teflon polytetra-fluoroethylene (PTFE) syringe filters and injected directly into the HPLC column. Flavonoid aglycones were separated on a C_{18} column (250×4.6 mm, 5 μm, Inertsil ODS-3; GL Sciences, Tokyo, Japan) by HPLC (Shimadzu, Kyoto, Japan) equipped with a photodiode array (PDA) detector. Elution was performed using a binary gradient of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) according to following program: 0 min, 95% A/5% B; 30 min, 45% A/55% B; 45 min, 35% A/65% B; 50 min, 0% A/100% B; 60 min, 0% A/100% B; 62 min, 95% A/5% B; and 70 min, 95% A/5% B. The flow rate was 1.0 mL/min and the column temperature was 40°C. The ultraviolet-visible (UV-vis) detector wavelength was set to 364 nm. Calibration curves were drawn for quantification by plotting five concentrations of the standards.

Extraction and analysis of anthocyanins Anthocyanin extraction was performed according to method of Park *et al*. (8). Anthocyanin was separated in a C_{18} column (250×4.6 mm, 5 μm, Inertsil ODS-3; GL Sciences) by HPLC as described above. Elution was performed using a binary gradient of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) according to following program: 0 min, 95% A/5% B; 20 min, 75% A/25% B; 22 min, 0% A/100% B; 32 min, 0% A/100% B; 34 min, 95% A/5% B; and 44 min, 95% A/5% B. The flow rate was 1.0 mL/min and the column temperature was 40°C. The UV-vis detector wavelength was set to 520 nm. Calibration curves were drawn for quantification by plotting five concentrations of the standards.

Extraction and analysis of phenolic acid Methanol-soluble (free and esterified forms) and -insoluble (bound form) phenolic acids were extracted according to the procedure described by Park *et al*.

(11). 3,4,5-Trimethoxycinnamic acid was added as an IS. For derivatization, MTBSTFA containing 1% TBDMCS and pyridine were added to the dried extracts followed by incubation at 60°C for 30 min at a mixing frequency of 1,200 rpm using a thermomixer comfort (Eppendorf AG, Hamburg, Germany). Each derivatized sample (1 μL) was injected into a 7890A gas chromatograph (Agilent Technologies) using a 7683B autosampler (Agilent Technologies) with a split ratio of 10, and separated on a 30 m×0.25-mm i.d. fused silica capillary column coated with 0.25-μm CP-SIL 8 CB low bleed (Varian Inc., Palo Alto, CA, USA). The injector temperature was 230°C and the flow rate of helium gas through the column was 1.0 mL/min. The temperature program was set to 150°C and maintained for 2 min followed by a 15°C/min oven temperature ramp to 320°C and 10 min hold. The column effluent was introduced into a Pegasus HT TOF mass spectrometer (LECO, St. Joseph, MI, USA). The transfer line and the ion-source temperatures were 250 and 200°C, respectively. The detected mass range was *m/z* 85-700, and the detector voltage was set to 1700 V. For quantification purposes, a standard stock solution of six phenolics (ferulic, *p*-coumaric, *p*-hydroxybenzoic, sinapic, syringic, and vanillic acids) and 3,4,5-trimethoxycinnamic acid used as an IS was prepared in methanol (100 μg/mL). Calibration samples, ranging from 0.01 to 10.0 μg, were prepared by mixing individual stock solutions of the six phenolic acid standards.

Metabolite profiling Polar metabolite extraction was performed as described previously (8). Ribitol was used as an IS. Methoxime (MO)-derivatization was performed by adding methoxyamine hydrochloride in pyridine and shaking at 30°C for 90 min. Trimethylsilyl (TMS) etherification was performed by the addition of MSTFA at 37°C for 30 min. A derivatized sample (1 μL) was separated on the same column as described above. The split ratio was set to 1:25 with an injector temperature of 230°C and a helium gas flow rate of 1.0 mL/min. The temperature program was set as follows: initial temperature of 80°C for 2 min followed by an increase to 320°C at 15°C/min and 10 min hold at 320°C. The transfer line and ion-source temperatures were 250 and 200°C, respectively. The scanned mass range was *m/z* 85-600 and the detector voltage was set to 1,700 V.

Statistical analysis The experiments were performed with three independent samples for biological replicates. Experimental data were analyzed by analysis of variance (ANOVA), and significant differences among the means were determined by Duncan's multiple-range test (SAS 9.2; SAS Institute, Cary, NC, USA). Quantification data were subjected to PLS-DA (SIMCA-P version 12.0; Umetrics, Umeå, Sweden) to evaluate differences among groups of multivariate data. The PLS-DA output consisted of score plots to visualize the contrast between samples and loading plots to explain the cluster separation. The quality of the PLS-DA model is depicted by the cross-validation parameters, R^2 and Q^2, representing the explained variance and the predictive capability of the model, respectively. The values of R^2 and Q^2 equating to -1 indicate an effective model. Permutation test (200 cycles) was conducted to assess the robustness of the PLS-DA model. Pearson correlation analysis was performed with the SAS 9.2 software package (SAS Institute, Cary, NC, USA). The correlation analysis was performed on the relative levels of 59 metabolites with standardization pre-processing. HCA and heatmap visualization of the correlation coefficient were performed using MultiExperiment Viewer version 4.4.0 software (http://www.tm4.org/mev/).

Table 1. Contents (μg/g on a dry weight basis) of carotenoids, flavonoids, and anthocyanidinsin color-fleshed sweet potatoes

	White	Orange	Purple
Carotenoids			
Lutein	0.27±0.01a[1)]	0.15±0.00b	0.28±0.05a
Zeaxanthin	0.03±0.00b	ND	0.11±0.03a
α-Carotene	0.01±0.00b	0.44±0.03a	ND
(all *E*)-β-Carotene	0.83±0.01c	68.74±0.41a	1.53±0.14b
(9*Z*)-β-Carotene	0.09±0.00b	1.45±0.03a	0.02±0.00c
(13*Z*)-β-Carotene	0.14±0.00b	22.64±0.60a	0.28±0.01b
Sum	1.37±0.03	93.41±1.01	2.22±0.21
Flavonoids			
Quercetin	19.83±5.47c	59.96±1.79b	388.85±11.34a
Myricetin	23.49±1.87c	39.78±2.01b	152.11±6.16a
Kaempferol	2.10±0.52c	18.89±1.39b	23.38±0.97a
Luteolin	ND	8.49±0.43b	15.17±0.74a
Sum	45.41±7.73	127.12±4.68	579.50±19.04
Anthocyanidins			
Cyanidin	ND	ND	408.35±31.98
Peonidin	ND	ND	319.06±78.26
Sum			727.42±86.93

[1)]Different letters represent significant (p<0.05) differences between means according to ANOVA combined with Duncan's multiple range test. Each value represents the mean±standard deviation (n=3). White, Yulmi; orange, Juhwangmi; purple, Sinjami; ND, not detectable.

Results and Discussion

Carotenoid, flavonoid, anthocyanin, and phenolic acid contents in color-fleshed sweet potatoes HPLC analyses were conducted using authentic standards to confirm carotenoid, flavonoid, and anthocyanin contents in three colored tuberous sweet potatoes. The quantitative results of these compounds from white-, orange-, and purple-fleshed varieties are shown in Table 1. Carotenoids were identified based on retention times of standards and compared with published data (9,12). Four carotenoids, such as lutein, zeaxanthin, and α- and β-carotene, were identified in white-fleshed sweet potatoes, but zeaxanthin and α-carotene were not detected in orange- or purple-fleshed sweet potatoes, respectively. The most abundant carotenoid in all tuberous extracts was β-carotene, including all-*trans* forms and isomers of *cis* β-carotene, which comprised >77% of total carotenoid content. In particular, β-carotene accounted for >99% of total carotenoid content in orange-fleshed sweet potatoes. As expected, total

Table 2. Contents (μg/g on dry weight basis) of soluble and insoluble phenolic acids in color-fleshed sweet potatoes

	Soluble			Insoluble			Total		
	White	Orange	Purple	White	Orange	Purple	White	Orange	Purple
p-OH	2.91±0.11b	5.06±0.17b	150.31±2.57a[1)]	2.60±0.03b	2.71±0.02b	88.24±6.87a	5.52±0.12b	7.77±0.15b	238.55±9.30a
Van	3.85±0.10b	4.42±0.12b	110.88±3.37a	3.62±0.32b	3.44±0.05b	36.55±1.36a	7.46±0.27b	7.86±0.10b	147.44±3.85a
Syr	1.78±0.01c	1.99±0.05b	2.07±0.02a	1.89±0.17a	1.77±0.03a	1.79±0.01a	3.67±0.17a	3.76±0.08a	3.86±0.03a
Cou	4.48±0.07c	8.74±0.61b	14.59±1.33a	2.97±0.17b	3.24±0.01a	3.48±0.16a	7.45±0.10c	11.98±0.62b	18.07±1.48a
Fer	9.61±0.41b	20.01±0.64b	223.66±12.93a	5.44±0.28b	4.55±0.10b	98.63±11.77a	15.05±0.65b	24.56±0.73b	322.29±23.55a
Sin	6.59±0.01c	8.56±0.57a	7.31±0.12b	6.73±0.01a	6.64±0.01a	6.75±0.12a	13.32±0.02c	15.20±0.57a	14.06±0.23b
Sum	29.22±0.29	48.77±0.49	508.82±17.49	23.26±0.87	22.36±0.11	235.44±11.07	52.48±0.82	71.13±0.59	744.26±28.31

[1)]Different letters represent significant (p<0.05) differences between means according to ANOVA combined with Duncan's multiple range test. Each value represents the mean±standard deviation (n=3). White, Yulmi; orange, Juhwangmi; purple, Sinjami; *p*-OH, *p*-hydroxybenzoic acid; Van, vanillic acid; Syr, syringic acid; Cou, *p*-coumaric acid; Fer, ferulic acid; Sin, sinapic acid.

carotenoid level was the highest in orange-fleshed sweet potatoes (93.41 μg/g) and the lowest in white-fleshed sweet potatoes (1.37 μg/g). Simonne *et al*. (13) reported a wide range in β-carotene content (1-190 μg/g) among various sweet potato breeding lines. Grace *et al*. (14) reported 1.0 and 253.3 μg/g β-carotene contents in purple- and orange-fleshed sweet potatoes, respectively. This variation in carotenoid content could be due to the differences in genotype, cultivation conditions, state of maturity, or storage and handling of the samples (15).

Flavonoid concentration was the highest in the purple variety (579.50 μg/g), followed by the orange- and white-fleshed sweet potatoes (127.12 and 45.41 μg/g, respectively). Quercetin was the most plentiful flavonoid in both orange- and purple-fleshed sweet potatoes, comprising 47 and 67% of total detected flavonoids, followed by myricetin (31 and 26%, respectively). However, the percentage of myricetin (51%) was slightly higher than that of quercetin (43%) in white-fleshed sweet potatoes. Kaempferol and luteolin were the least abundant flavonoids in all samples analyzed, and luteolin was not detected in white-fleshed sweet potatoes. The flavonoid composition observed in this study was consistent with a previous report that the low relative kaempferol accumulation in the leaves and tubers of sweet potatoes may result from conversion of kaempferol to quercetin and myricetin because dihydrokaempferol is a dihydroquercetin and dihydromyricetin precursor (16). Anthocyanins in sweet potatoes were investigated by analyzing their hydrolytic products because complex glycoslyation patterns make identifying individual anthocyanins difficult, even by liquid chromatography-MS analysis (17). Anthocyanins were detected only in purple-fleshed sweet potatoes, and the hydrolytic products included 408.35 μg/g cyanidin and 319.06 μg/g peonidin, respectively. Anthocyanin composition has been previously determined in purple-fleshed sweet potatoes by Grace *et al*. (14) and Lee *et al*. (18). In those studies, cyanidin and peonidin glycosides acylated with caffeic, *p*-hydroxybenzoic, and ferulic acids were the primary anthocyanin components identified in purple-fleshed sweet potatoes, revealing the wide range in the levels of these compounds among sweet potato varieties. Color variations in plants are primarily the result of various pigments, such as chlorophylls, carotenoids, and anthocyanins. Carotenoids primarily impart yellow, orange, and red colors, which were confirmed in the orange-fleshed sweet potatoes by their higher β-carotene level compared to that in the white- and purple-fleshed sweet potatoes. It is difficult to predict flavonoid content based on color alone because only a few hundred flavonoids appear in the colored state among the thousands of flavonoids in plants. Anthocyanins are the most intensely colored flavonoid pigments and appear as red, purple, or blue in sweet potatoes (16). According to Montilla *et al*. (19), sweet potato varieties can be classified into two groups based on the peonidin/cyanidin (p/c) ratio: cyanidin type (p/c <1.0), with a greater degree of blueness (blue-dominant group) and the peonidin type (p/c >1.0), with a greater degree of redness (red-dominant group). The purple-fleshed sweet potatoes (cv. 'Sinjami') investigated in this study were classified as the cyanidin type (p/c=0.78).

Phenolic acid levels in the methanol-soluble and -insoluble phenolic fractions of the sweet potatoes were determined by GC-TOFMS. As shown in Table 2, all samples contained six phenolic acids, including *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic, and sinapic acid, which were previously identified as phenolics by our group using GC-TOFMS (8,20). However, the levels of these compounds in sweet potatoes with different tuber colors have not been previously evaluated. The total levels of the six phenolic acids in purple-fleshed sweet potatoes (744.26 μg/g) were >10-fold higher than those in the white- and orange-fleshed sweet potatoes (52.48 and 71.13 μg/g, respectively). The quantity and composition of individual phenolic acids in the color-fleshed sweet potatoes varied from 3.67 to 322.29 μg/g. Most of the phenolic acids in purple-fleshed sweet potatoes were *p*-hydroxybenzoic, vanillic, and ferulic acids, which accounted for >95% of total content, whereas ferulic and sinapic acids represented the greatest proportion in the white- and orange-fleshed sweet potatoes (54.05 and 55.89%, respectively). Padda and Picha (21) and Grace *et al*. (14) showed that chlorogenic, caffeic, and dicaffeoylquinic acids are the major phenolic acids in colored sweet potato tubers, as identified by HPLC.

Classification of sweet potato varieties by GC-TOFMS-based metabolic profiling and PLS-DA Untargeted metabolomic approaches using GC-MS are one of the most useful techniques to explore metabolic

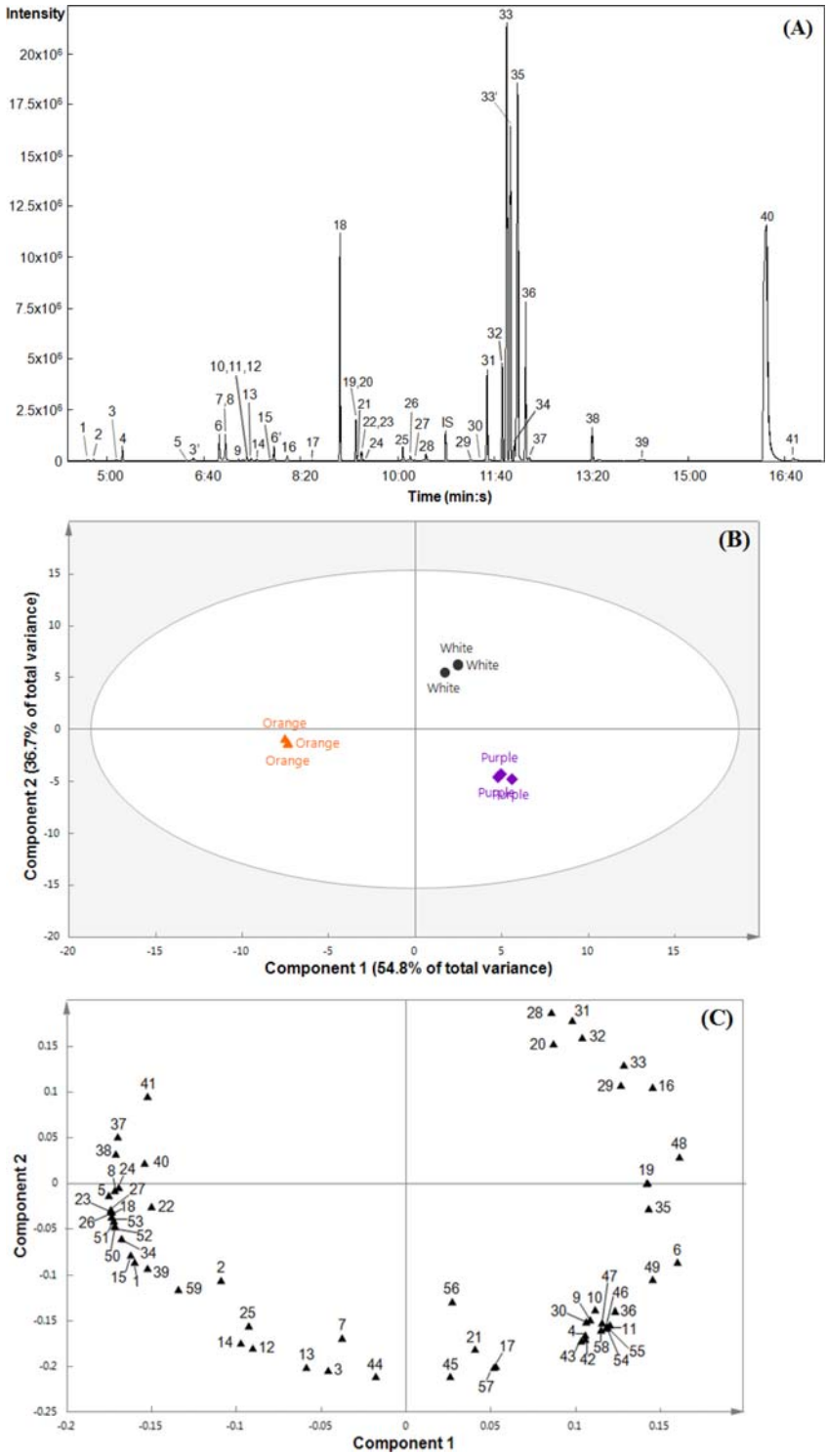

Fig. 1. (A) Selected ion chromatograms of hydrophilic metabolites extracted from purple-fleshed sweet potatoes (cv. Sinjami) as MO/TMS derivatives separated on a 30 m×0.25-mm i.d. fused silica capillary column coated with 0.25 μm CP-SIL 8 CB low bleed. Score (B) and loading (C) plots of principal components 1 and 2 of the PLS-DA results obtained from data on 59 metabolites of color-fleshed sweet potatoes. 1, pyruvic acid; 2, lactic acid; 3, valine; 4, alanine; 5, glycolic acid; 3', valine; 6, serine; 7, ethanolamine; 8, glycerol; 9, isoleucine; 10, proline; 11, nicotinic acid; 12, glycine; 13, succinic acid; 14, glyceric acid; 15, fumaric acid; 6', serine; 16, threonine; 17, β-alanine; 18, malic acid; 19, salicylic acid; 20, aspartic acid; 21, methionine; 22, pyroglutamic acid; 23, 4-aminobutyric acid; 24, threonic acid; 25, glutamic acid; 26, phenylalanine; 27, xylose; 28, asparagine; 29, glutamine; 30, shikimic acid; 31, citric acid; 32, quinic acid; 33, fructose; 33', fructose; 34, galactose; 35, glucose; 36, mannose; 37, mannitol; 38, inositol; 39, tryptophan; 40, sucrose; 41, trehalose; 42, quercetin; 43, myricetin; 44, kaempferol; 45, luteolin; 46, cyanidin; 47, peonidin; 48, lutein; 49, zeaxanthin; 50, 13-*cis*-β-carotene; 51, *α*-carotene; 52, β-carotene; 53, 9-*cis*-β-carotene; 54, *p*-hydroxybenzoic acid; 55, vanillic acid; 56, syringic acid; 57, *p*-coumaric acid; 58, ferulic acid; 59, sinapic acid; IS, internal standard (ribitol).

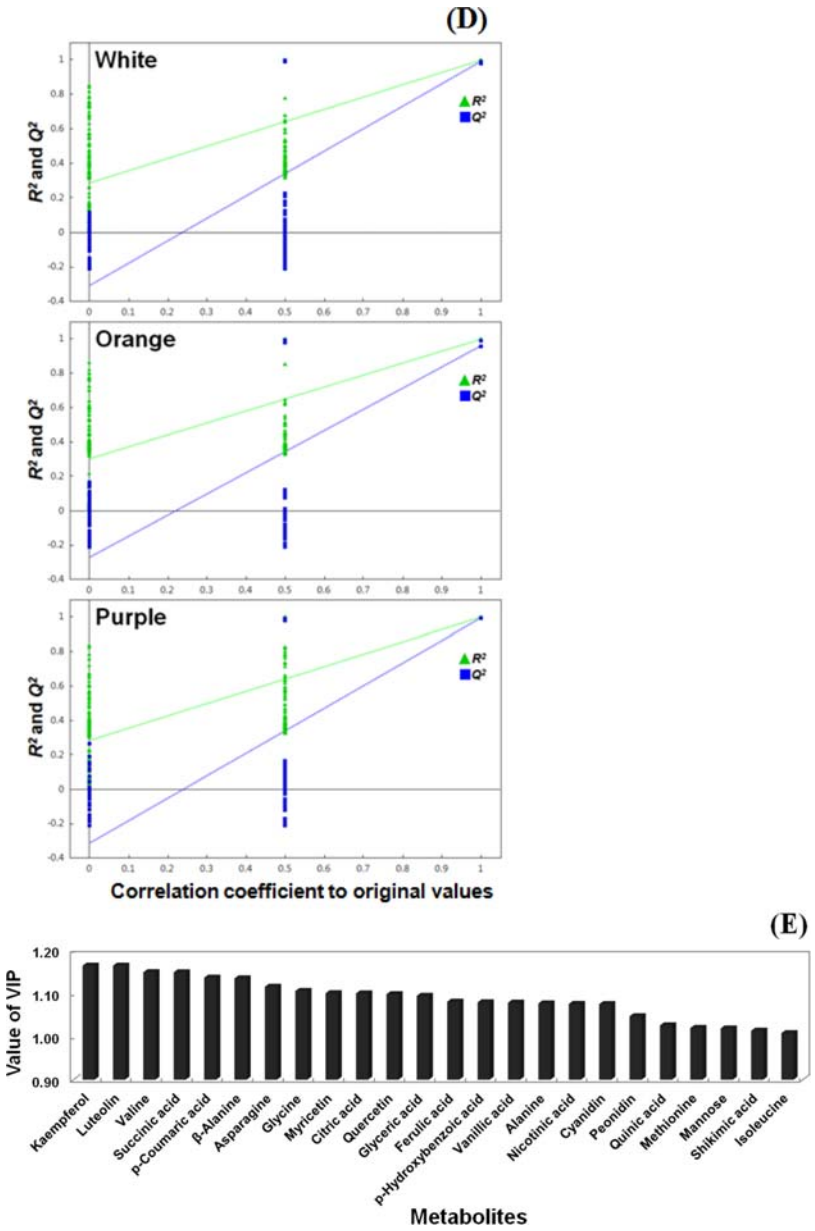

Fig. 2. Continued. (D) Validation plots of the PLS-DA model for three colored sweet potatoes using a permutation test with 200 random permutations (E) The top 24 metabolites ranked by variable importance in the prediction (VIP) value obtained from the PLS-DA analysis with data on 59 metabolites of color-fleshed sweet potatoes.

differences among genotypes (22) and to identify associations between phenotypic traits and primary metabolites (6). In this study, GC-MS-based metabolite profiling was conducted to assess the variations in polar primary metabolites in the different-colored sweet potatoes by GC-TOFMS. ChromaTOF software was used to assist with peak location and for automated deconvolution of reference mass spectra. The peaks were identified by comparison with reference compounds and an in-house library (8). In addition, several metabolites were identified by directly comparing their sample mass chromatograms with those of commercially available standard compounds, which were obtained by a similar MO/TMS derivatization and GC-TOFMS analysis. In total, 41 metabolites, including 17 amino acids, 13 organic acids, 7 sugars, 3 sugar alcohols, and 1 amine were detected in the color-fleshed sweet potatoes (Table 3). The corresponding GC-MS retention times and selected ions for quantification are illustrated in Table 3 and Fig. 2A. Quantification of all analytes was based on the peak area ratio relative to that of the internal standard. The quantitative data for these 41 primary metabolites and 18 bioactive secondary compounds (carotenoids, flavonoids, anthocyanins, and phenolic acids) identified in this study were subjected to PLS-DA to detect differences in the metabolite profiles among the varieties. The data file was scaled with unit variance scaling before all of the variables were subjected to PLS-DA. PLS-DA has been used to identify metabolic differences among varieties in diverse plant samples with

Table 3. Metabolites identified in GC-TOFMS chromatograms of purple-fleshed sweet potatoes (cv. Sinjami) extracts

No.[1)]	Compound	RT[2)]	RRT[3)]	Selected ion for quantification[4)]
1	Pyruvic acid	4.67	0.431	174
2	Lactic acid	4.77	0.440	147
3	Valine	5.16	0.477	146
4	Alanine	5.27	0.486	116
5	Glycolic acid	6.37	0.588	147
6	Serine	6.93	0.640	116
7	Ethanolamine	7.02	0.648	174
8	Glycerol	7.04	0.650	147
9	Isoleucine	7.27	0.671	158
10	Proline	7.35	0.679	142
11	Nicotinic acid	7.40	0.683	180
12	Glycine	7.41	0.684	174
13	Succinic acid	7.48	0.691	147
14	Glyceric acid	7.59	0.700	147
15	Fumaric acid	7.83	0.722	245
16	Threonine	8.11	0.749	219
17	β-alanine	8.53	0.788	174
18	Malic acid	9.01	0.832	147
19	Salicylic acid	9.28	0.857	267
20	Aspartic acid	9.29	0.857	100
21	Methionine	9.34	0.862	176
22	Pyroglutamic acid	9.38	0.866	156
23	4-aminobutyric acid	9.41	0.869	174
24	Threonic acid	9.56	0.882	147
25	Glutamic acid	10.09	0.932	246
26	Phenylalanine	10.22	0.944	218
27	Xylose	10.31	0.952	103
28	Asparagine	10.50	0.969	116
IS	Ribitol	10.83	1.000	217
29	Glutamine	11.27	1.040	156
30	Shikimic acid	11.42	1.054	204
31	Citric acid	11.55	1.066	273
32	Quinic acid	11.81	1.090	345
33	Fructose	11.89	1.097	103
34	Galactose	12.02	1.110	147
35	Glucose	12.07	1.115	147
36	Mannose	12.21	1.127	147
37	Manitol	12.27	1.133	319
38	Inositol	13.35	1.233	305
39	Tryptophane	14.20	1.311	202
40	Sucrose	16.36	1.510	217
41	Trehalose	16.81	1.552	191

[1)]Numbers represent the compound index for the chromatogram peaks shown in Fig. 2.
[2)]Retention time (min).
[3)]Relative retention time (retention time of the analyte/retention time of the IS).
[4)]Specific mass ion used for quantification.

the supervised pattern recognition method (22,23). The PLS-DA results demonstrated a lack of significant variance within the same variety (Fig. 2B). The quality of the model was described using R^2 and Q^2 values. R^2 is the proportion of variance in the data explained by the model and indicates goodness of fit. Q^2 is the proportion of variance in the data predicted by the model and indicates predictability. $Q^2$is also called cross-validated R^2. A Q^2 value >0.9 indicates that the model has excellent predictive ability (24,25). The model had an R^2 value of 0.997 and a Q^2 value of 0.99. The two highest-ranking components accounted for 91.5% of the total variance within the data set. The first components accounted for 54.8% of the total variation and resolved the measured composition profiles of orange-fleshed sweet potatoes from the other two varieties. The corresponding loading was mainly positive for lutein, serine, threonine, and zeaxanthin (Fig. 2C). The loading plots also indicated that higher levels of glycolic acid, phenylalanine, 4-aminobutyric acid, malic acid, and α- and β-carotenes were present in orange-fleshed sweet potatoes than those in the other two varieties. In addition, white-fleshed sweet potatoes were separated from orange- and purple-fleshed sweet potatoes by the second components, which accounted for 36.7% of the total variation. The corresponding loading was positive for asparagine and citric and quinic acids but negative for all carotenoids, flavonoids, anthocyanins, and phenolic acids with the exception of lutein, indicating that orange- and purple-fleshed sweet potatoes had higher bioactive secondary metabolite content than that of white-fleshed sweet potatoes. Additionally, statistical significance of the PLS-DA model was evaluated with permutation testing (Fig. 2D). In the permutation tests with 200 random permutations, all R^2 and Q^2 values were higher than 0.9, revealing great predictability and goodness of fit.

The contribution of variables in the projection was explained using the significance of the variable in the projection (VIP) value. VIP is a weighted sum of squares of the PLS weight, and a value >1 is generally used as a criterion to identify the most important variables in a model (25). Among the metabolites identified, 24 had a significant VIP value (>1). The variable that played the greatest role discriminating between the metabolic profiles of the color-fleshed sweet potatoes was kaempferol, followed by luteoin, valine, and succinic and *p*-coumaric acids (Fig. 2E). A heat map was created to visualize the metabolic differences among samples (Fig. 3A), in which all samples were correctly classified according to their genotype. The heat map showed differences in the relative metabolite levels of the three sweet potato varieties. Notably, the orange- and purple-fleshed sweet potatoes had higher levels of sugars, sugar alcohols, and secondary metabolites compared with those in the white-fleshed sweet potatoes. Far and Taie (26) reported that phenolic compounds accumulate due to the increased sugar or sorbitol levels in sweet potatoes. Lee *et al*. (27) also reported that *Aloe vera*, which contains very high antioxidant activity, has relatively high sugar and anthra-quinone derivative (as phenolic metabolites) contents that were modified by adding a sugar molecule. Our study is the first to demonstrate compositional differences in the core primary metabolite profiles and major secondary metabolites in color- fleshed sweet potatoes, suggesting that the primary metabolite profiling approach using chemometrics is useful for tracking possible metabolic links.

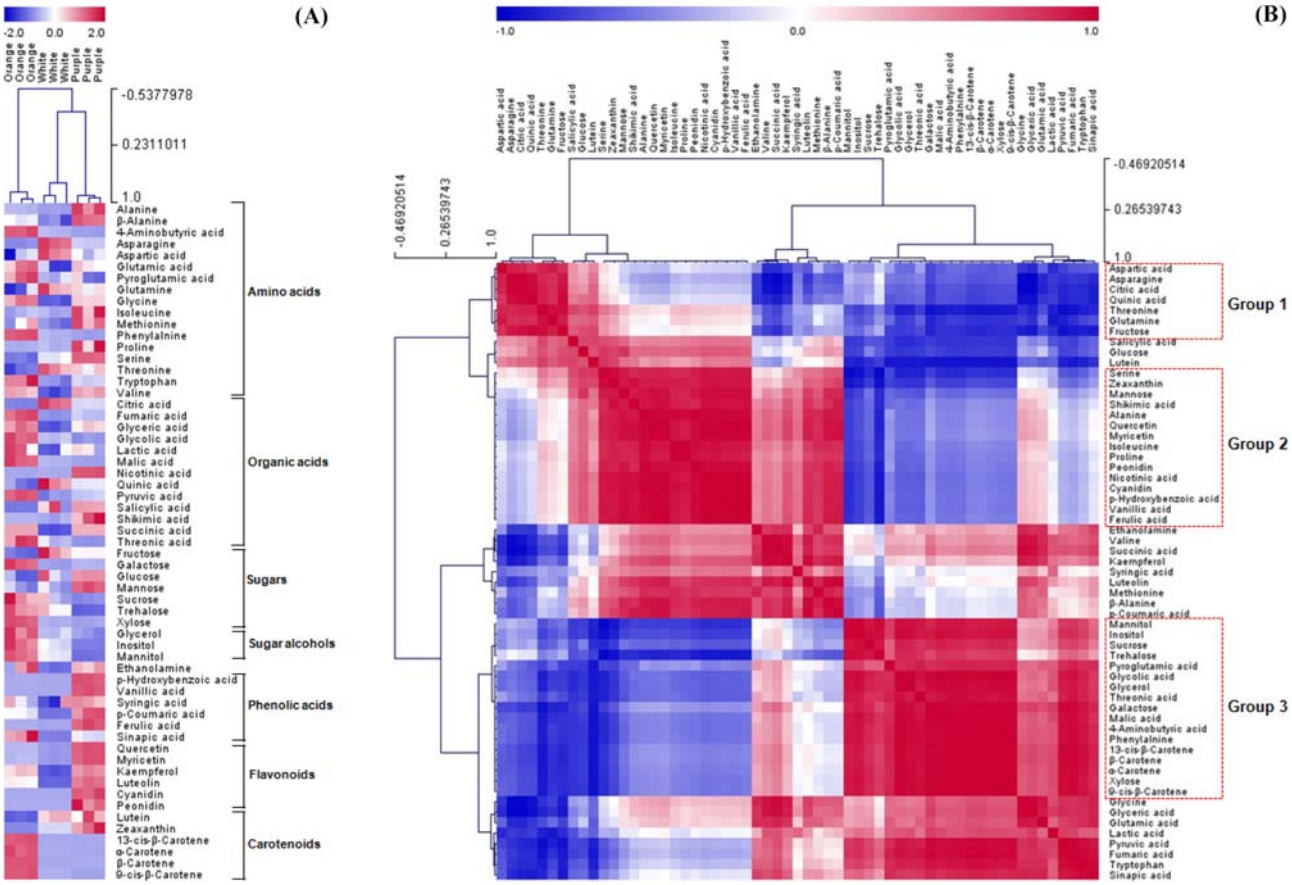

Fig. 3. (A) Heat map representing differences in relative metabolite levels of three sweet potato varieties. Red or blue indicates that the metabolite content is decreased or increased, respectively. (B) Correlation matrix and cluster analysis of results obtained from data on 59 metabolites of color-fleshed sweet potatoes. Each square indicates the Pearson's correlation coefficient of a pair of compounds, and the value for the correlation coefficient is represented by the intensity of the blue or red color, as indicated on the color scale. Hierarchical clusters are represented by a cluster tree.

Kim *et al*. (7) revealed the metabolic networks connecting primary and secondary metabolism in rice grains using hydrophilic metabolite profiling combined with chemometrics.

Correlations between levels of metabolites in the color-fleshed sweet potatoes Correlation analysis is useful to determine the strength of a relationship between two quantitative samples and can be used to detect associations between metabolites in a biological system. Intrinsic fluctuations in a metabolic system induce a characteristic pattern of interdependencies between metabolites that can be exploited using a comparative correlation analysis (28). Pearson's correlation analysis and HCA were performed to examine the detailed relationships between the levels of 59 sweet potato metabolites. As results, a significant positive correlation was detected between phenylalanine and tryptophan (r=0.9284, p<0.0001), which are biologically linked aromatic amino acids (6). Secondary metabolites, such as phenolic acids, flavonoids, and anthocyanins, are produced in the shikimate pathway. In our results, shikimic acid content was positively correlated with quercetin (r=0.9402, p=0.0002), myricetin (r=0.9447, p<0.0001), cyanidin (r=0.9459, p<0.0001), *p*-hydroxybenzoic acid (r=0.9183, p=0.0005), vanillic acid (r=0.9306, p=0.0003), *p*-coumaric acid (r=0.9016, p=0.0009), and ferulic acid (r=0.9444, p<0.0001) contents. The HCA results obtained from the Pearson's correlation coefficients revealed three major metabolite clusters, which are marked with a dotted box in Fig. 3B. Aspartic acid, asparagine, citric acid, quinic acid, threonine, glutamine, and fructose clustered within Group 1 and were generally negatively correlated with the other metabolites. Group 3 metabolites also revealed a robust negative correlation with Group 1 and Group 2 metabolites. Group 1, Group 2, and Group 3 metabolites were identified at high levels in the white-, purple-, and orange-fleshed sweet potatoes, respectively (Fig. 3A). These results are completely consistent with the findings from the PLS-DA loading plots (Fig. 2C), indicating that PLS-DA is useful for visualizing complex data.

In conclusion, we identified the core primary metabolites, including amino acids, organic acids, sugars, and sugar alcohols and quantified the health-beneficial secondary metabolites (carotenoids, flavonoids, anthocyanins, and phenolic acids) in color-fleshed sweet potatoes (white, orange, and purple). The carotenoid content was considerably higher in orange-fleshed sweet potatoes than that in

the other varieties, wherein β-carotene was the most plentiful, and anthocyanins were detected only in purple-fleshed sweet potatoes. The total levels of four flavonoids and six phenolic acids were relatively higher in purple-fleshed sweet potatoes than those in the other varieties. These significant variations in bioactive compounds among sweet potato varieties indicate that composition profiles can potentially be manipulated through conventional breeding programs and/or molecular biotechnological approaches to develop new sweet potato cultivars or new crops with increased health benefits. Our metabolite profiles were applied to data mining processes, including PLS-DA, Pearson's correlation analysis, and HCA, which helped identify significant compositional differences and relationships among primary and secondary metabolites in the color-fleshed sweet potatoes. A strong correlation was detected between metabolites that participate in closely related pathways. All multivariate analyses showed a similar sweet potato classification based on genotype, in which orange- and purple-fleshed sweet potatoes, containing high levels of secondary metabolites, had higher sugar and sugar alcohol levels than those in white-fleshed sweet potatoes. Our results suggest the usefulness of GC-TOFMS-based metabolite profiling, combined with chemometrics, as a tool for determining phenotypic variations and identifying links between primary and secondary metabolism.

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