농학석사학위논문

GC-MS/MS 의 targeted metabolite profiling 을 이용한 감귤류 과일의 비교 대사체학

A comparative metabolomics of various citrus fruits using targeted metabolite profiling on GC-MS/MS

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농생명공학부 응용생명화학전공

이 강 헌

A Dissertation for the Degree of Master of Science

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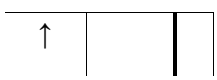
Kanghyun Lee

Applied Life Chemistry Major

Department of Agricultural Biotechnology

Seoul National University

외표지: 책등(측면



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지도교수 김 정 한

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2020년 1월

위 원 장 신 찬 석 (인)

부 위 원 장 김 정 한 (인)

위 원 오 기 봉 (인)

Abstract

The purpose of this study was to isolate the flesh and peel of Miyamoto(*Citrus unshiu Marcow*), Hanlabong(*Citrus hybrid Shiranuhi*), Redhyang(*Citrus hybrid Kanpei*), Hwangkeumhyang(*Citrus hybrid Beni Madonna*), among citrus fruits, one of the most commonly consumed fruits in Korea, and then compare the metabolites with metabolomics, separately. Fruits were analyzed in GC-MS/MS using a derivatization method that improves and allows better separation. Based on the analyzed data, the primary screening was selected using score scatter plot of the Principal Component Analysis (PCA) and ANOVA, which is a statistical tool, and then more precisely using the Partial Least Squares Discriminant Analysis (PLS-DA). The substance which specified the sample of each citrus fruits were identified. For Miyamoto, n-acetylneuraminic acid, glucono-1,5-lactone and inositol in flesh, In addition methionine and tyramine in the skin were identified as specific substances. In case of Hallabong, gluconic acid, 2-deoxy-glucose and 4-hydroxyphenylpyruvic acid in flesh, o-acetylserine, pentadecanoic acid, hippuric acid in peel, tyramine, octopamine and coniferyl alcohol in the flesh of Hwangkeumhyang, and xamic acid, putrescine and xanthosinein the peel were identified as the specific substances. According to Redhyang adenine, 2-amino-octanoic acid and valproic acid were in flesh, cystathionine, spermidine and thymidine in the skin were identified.

Key words: ANOVA, Derivatization, GC-MS/MS, Hwangkeumhyang, Hanlabong Metabolomics, Miyamoto, PCA, PLS-DA, Redhyang

***Student number*: 2010-21252**

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List of Abbreviations

GC/MS	Gas chromatography mass spectrometry
MS/MS	tandem mass spectrometry
MRM	multiple reaction monitoring
MSTFA	N-Methyl-N-trimethylsilyltrifluoroacetamide
PCA	principal component analysis
PLS-DA	partial least squares discriminant analysis

Introduction

Tangerines

Fruit is one of the most basic and consumed important foods in our lives. In modern society, the agricultural industry also breeds high sugar and functional crops to produce high value added fruits such as bioactive and phenolic compounds containing antioxidant ingredients (Suh et al., 2013). In the food industry, the rapid and simultaneous analysis of healthy components, as well as monosaccharides and polysaccharides and oligosaccharides in fruits will be very useful for quality improvement, inspection and management of products. Thus, in addition to the sugars and organic compounds that have been analyzed a lot in fruit, we analyzed them to see what ingredients exist. Four fruits tangerines Miyamoto(*Citrus unshiu Marcow*), Hanlabong(*Citrus hybrid Shiranuhi*), Redhyang(*Citrus hybrid Kanpei*) and Hwangkeumhyang(*Citrus hybrid Beni Madonna*) which were widely consumed in Korea, and there were one of the fruits that can be eaten both flesh and peel. In addition, flesh can be eaten directly or with juice, and the peel can be consumed as a traditional tea (Lunzhao yi et al., 2015). Also they contain pytochemical and large amounts of vitamin c (Yoo et al., 2005). Thus, the tangerines were analyzed using GC-MS/MS through derivatization method. Especially, derivatization allows us to analyze more components in less time. Knowing the type and amount of ingredients in each fruit could help us to solve the aforementioned problems.

Metabolomics

Metabolomics is the large-scale scientific study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms. Collectively,

these small molecules and their interactions within a biological system are known as the metabolome. Metabolomics aims at the comprehensive and quantitative analysis of various kinds of metabolites in biological samples. In addition, it aims to provide a global snapshot of all small-molecule metabolites in cells and biological fluids without the observed bias inherent in more focused metabolic studies (Katja Dettmer et al., 2006). Efficiently forming biologically relevant conclusions from a given metabolic dataset actually requires a special form of data analysis. One approach to finding meaning in metabolic datasets involves Multivariate Analysis (MVA) methods, such as partial least squares methods for Principal Component Analysis (PCA) and Latent Structure (PLS), where they contribute most to the transformation or separation Spectral features are identified for further analysis (Worely et al., 2013).

Principal Component Analysis (PCA) and Partial least squares Discrminant Analysis (PLS-DA) for statistical tool

Principal component analysis (PCA) is a multivariate procedure that simplifies and describes interrelationships among multiple dependent variables (Lawless and Heymann 2010). Partial least squares regression (PLS regression) is a versatile algorithm that can be used for differential variable selection as well as predictor and technical modeling. However, the user needs to optimize various parameters before reaching a reliable and valid result. Over the past two decades, PLS-DA has been very successful in modeling high-level data sets for various purposes, for example, product certification in food analysis, disease classification in medical diagnostics, and evidence analysis in forensic science (Loong et al., 2018)

Materials and Methods

Chemicals and reagents

Analytical standards for Adding to metabolic methods in GC-MS/MS

11-eicosenoic acid (Purity : 99%), campesterol (Purity : 65%), erythrose (Purity : 75%), lanosterol (Purity : 56%), methyl arachidonate (Purity : 99%), sitosterol (Purity : 95%) and traicontanol (Purity : 99%) were purchased from Sigma (Saint Louis, MO). cholestanol (Purity : 94.2%), 10-heptadecenoic acid (Purity : 99%), stigmasterol (Purity : 98.3%) and tetracosanoic acid (Purity : 99.5%) were purchased from Supelco (Darmstadt, Germany). Norleucine (Purity : 99%), salicylic acid (Purity : 99%), tetracosanoic acid (Purity : 99.5%) and turanose (Purity : 98%) were purchased from Honeywell (Charlotte, NC), benzylpalmitamide (Purity : 98%) was purchased from Caymen chemical (Ann arbor, MI). ribitol (Purity : 99%) was purchased from Wako (Osaka, Japan)

Internal Standard

Ribitol (Wako, Japan)

AART Standards

Qualitive Retention Time Index Std (RESTEK)

Solvents

Acetonitrile (Merck, Germany), methanol (Merck, Germany), pyridine (Sigma-aldrich, India), deionized water

Derivatization reagents

MSTFA + 1% TMCS (Thermo, Korea), Methoxyamine hydrochloride (Aldrich, UK)

Appratus

Freeze dryer with shell freezer FDS8508 (Ilshin, Korea),

Ultra-pure water was provided by Evoqua Labostar 7 TWF (Simens, German),

Thermomixer C Shaker (Eppendorf, Germany),

Centrifuge micro 17TR ((Hanil, Korea), Voltexer (Scientific, NY).

Samples

Tangerines (Miyamoto, Redhyang, Hwangkeumhyang, Hanlabong),

Derivatization for analaysis using GC-MS/MS

Derivatization is the process by which a compound is chemically changed to produce a new compound with properties that are more suitable for a particular analytical method, such as GC analysis (Kristi Sellers, 2010). Compounds with low volatility and thermal stability will exhibit reproducible peak areas, heights, and shapes (K.Sellers, 2010). The most common way to derivatize polar compounds containing functional groups, such as –OH, -SH, or –NH, is to add a trimethylsilyl (TMS) group, and form TMS ethers, TMS sulfides or TMS amines, respectively (J.Gullberg et al, 2004).

TMS derivatization and methoximation for analaysis using GC-MS/MS

One hundred milligrams of homogenized sample was extracted with 1.5mL of 80% methanol containing 200ng/ml of ribitol (internal standard). After 3 minutes of vortexing, the samples were centrifuged at 13,000 rpm for 3 minutes and 100 μL of supernatant was transferred to new 2mL eppendorf tube. The extract in the tubes were dried unsing speed vac at -80℃. The dried samples were oximated with 40 μL of methoxyamine hydrochloride dissolved in pyridines (20mg/mL) and incubated in thermomixer for 90minutes (1250 rpm, 37℃). Then 70 μL of MSTFA + 1% TMCS was added and incubated in thermomixer for 30minutes (1250 rpm , 37℃).

Table 1. Derivatization standards

Name
11-eicosenoic acid
Tetracosanoic acid
Campesterol
Lanosterol
Norleucine
Pentadecenoic acid
Turanose
Triacontanol
Erythrose
10-heptadecenoic acid
Benzylpalmitamide
Stigmasterol
Salicylic acid
Methyl-arachidonate
Cholestanol
Sitosterol

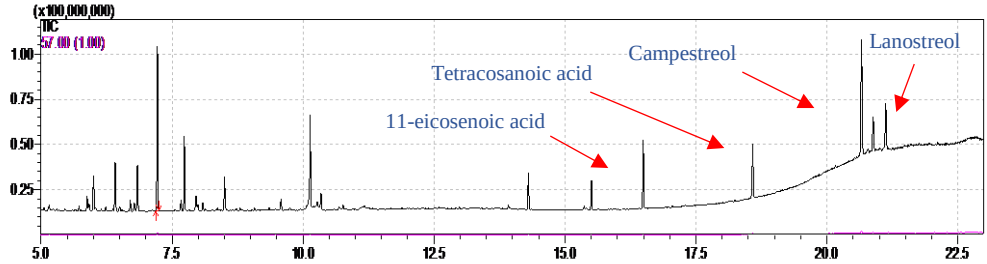
Optimization of standards for addition to metabolic methods in GC-MS / MS

Shimadzu "Smart Database" is a database file for creating method files using the "Smart MRM" function. I used the function to register the retention index in the database file. Smart MRM technology automatically generates methods with optimized measurement times for each component based on Smart databases. The AART (Automatic Retention of Retention Time) function integrated into the system provides a very accurate estimation of the retention time.

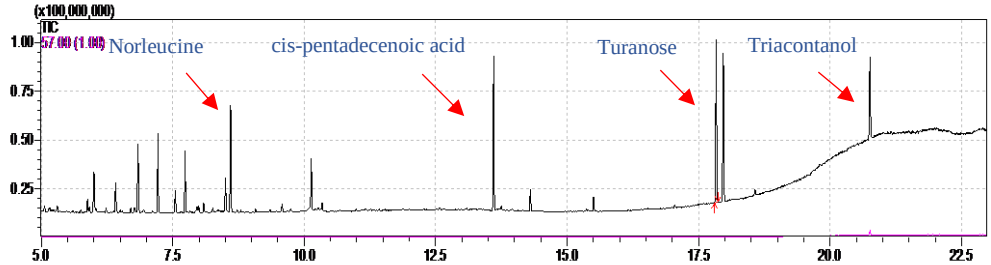
The "MRM Optimization Tool" automates the process by collecting product ion scan data and finding the optimal collision energy for each conversion. When the setting is completed, the conversion is registered in one of the Shimadzu “Smart Database” files and an MRM method is created using Smart MRM. Thus, after derivatization of 16 standards, each material is scanned and identified (Figure 1). And after confirming the MRM condition (Table 2) of the standard product, the result of optimization was added to the method.

Figure 1. TIC choromatogram of Optimization standards group (A),(B),(C),(D)

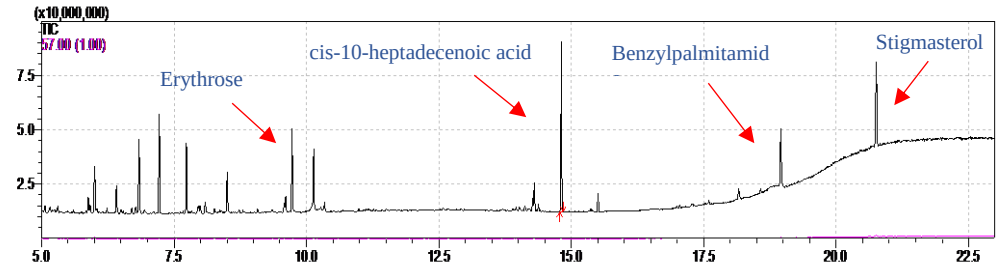
(A)


(x100,000,000)
TIC
57.00 (1.00)
Tetracosanoic acid
Campestreol
Lanostreol
11-eicosenoic acid

(B)


(x100,000,000)
TIC
57.00 (1.00)
Norleucine
cis-pentadecenoic acid
Turanose
Triacontanol

(C)


(x10,000,000)
TIC
57.00 (1.00)
Erythrose
cis-10-heptadecenoic acid
Benzylpalmitamid
Stigmasterol

(D)

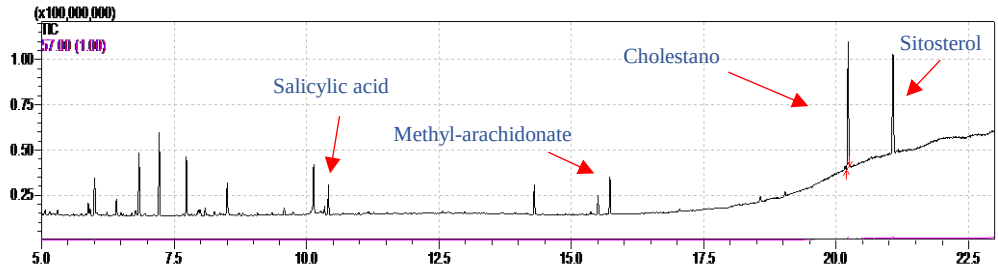

(x100,000,000)
TIC
57.00 (1.00)
Salicylic acid
Methyl-arachidonate
Cholestano
Sitosterol

Table 2. MRM Conditions of Optimization standard to add Metabolomics method

Name	Precursor ion	Fragment ion 1	CE	Fragment ion 2	CE
11-eicosenoic acid	367.35	75.10	15V	131.20	15V
Tetracosanoic acid	425.45	75.10	15V	131.10	15V
Campesterol	343.40	95.30	15V	121.30	15V
Lanosterol	498.50	109,20	15V	187.30	15V
Norleucine	158.15	73.10	15V	102.10	15V
Pentadecenoic acid	297.15	75.10	15V	131.10	15V
Turanose	307.20	73.10	15V	217.20	15V
Triacontanol	495.00	97.20	15V	75.10	15V
Erythrose	205.00	73.10	15V	147.10	15V
10-heptadecenoic acid	117.00	75.10	15V	60.10	15V
Benzylpalmitamide	149.00	106.10	15V	91.10	15V
Stigmasterol	129.00	55.10	15V	73.10	15V
Salicylic acid	267.15	73.00	15V	209.00	15V
Methyl-arachidonate	150.20	80.10	15V	77.10	15V
Cholestanol	215.25	159.20	15V	145.20	15V
Sitosterol	396.00	145.10	15V	171.30	15V

Sample preparation

Fresh fruit samples were purchased from the open market in Seoul, Korea. All fruits were separated flesh from peel, then chopped. Flesh and peel were freeze dried(-80℃) immediately. After that, the flesh and peel were homogenized with a mortar.

Figure 2. Samples of tangerine (A) Miyamoto (B) Hanlabong (C) Hwangkeumhyang (D) Redhywang

(A)

(B)

(C)

(D)

Method validation

Retention time adjustment by AART in GC-MS/MS

The AART (Automated Adjusted Rention Time) function is capable of simultaneously adjusting the rention times of target compounds based on liner retention indices, which are constants for a given column phase and GC parameter (Kovats 1958)

Retention index for a normal alkane is equal to its number of carbons multiplied by 100. For example, *n*-dodecane (*n*-C12H26) has *I*= 1200 and *n*-tridecane (*n*-C13H28) has *I*= 1300. A compound with a retention index of 1225 elutes between *n*-dodecane and *n*-tridecane, but closer to *n*-dodecane under the same instrumental conditions and column (Rood 2007). Retenion indices are useful when comparing relative elution orders of various compounds for a given column and conditions. Also, retention indices are good for comparing the retention behavior of two columns with the same description used under the same conditions (Rood 2007):

$$I_T = 100\left[\frac{\mathrm{t_r(x)} - \mathrm{t_r}(y)}{\mathrm{t_r(x)} + \mathrm{t_r}(y)}\right] + 100\mathrm{y}$$

t_r= retention time

X = compound of interest

Y = normal alkane with y carbon atoms eluting before compound x

Z = normal alkane with z carbon atoms eluting before compound x

.

Analytical instruments and conditions

GC-MS/MS condition

Shimadzu GCMS-TQ8040 was used for GC-MS/MS analysis. The analytical column was BPX-5 (30 m x 0.25 mm id., 0.25 μm df, TRAJAN, Australia). Injection temperature was 250 ℃ and injection mode was split mode. Split ratio was 30:1 and the column flow was 1.14 mL/min. The injection volume was 2 μL. The oven temperature programs was initialized at 60 ℃ (held for 2min), increased to 320 ℃ at 10 ℃/min (held for 15min). Total run time was 43 min. The ion source temperature was 200 ℃ and transfer line temperature was 280 ℃. The electron ionization energy was -70eV.

Table 3. List of total compounds : TMS derivatization and methoximation for analysis using GC-MS/MS

Compound name	Retention Index	Classification
ethylene glycol	975	alcohols
Dimethylglycine	984	amino acids
Glyoxylic acid	986	organic acids
2-Aminoethanol	1023	alcohols
Pyruvic acid	1046	organic acids
Lactic acid	1051	organic acids
2-Hydroxyisobutyric acid	1052	organic acids
Caproic acid	1069	fatty acids
Glycolic acid	1069	organic acids
Alanine	1095	amino acids
2-Ketobutyric acid	1096	organic acids
2-Keto-isovaleric acid	1102	organic acids
Hydroxylamine	1105	inorganic compounds
Norvaline	1109	amino acids
Glycine	1116	amino acids
2-Hydroxybutyric acid	1117	organic acids
Oxalic acid	1126	organic acids
Sarcosine	1131	amino acids
2-Aminoisobutyric acid	1135	amino acids
3-Hydroxypropionic acid	1135	organic acids
3-hydroxypyridine	1143	pyridines
Valproic acid	1145	fatty acids
3-Hydroxyisobutyric acid	1149	organic acids
2-Hydroxyisovaleric acid	1151	organic acids
3-Hydroxybutyric acid	1152	organic acids
4-hydroxypyridine	1159	pyridines
2-Aminobutyric acid	1164	amino acids
2-Ketoisocaproic acid	1171	organic acids
3-Methyl-2-oxovaleric acid	1172	organic acids
2-Methyl-3-hydroxybutyric acid	1184	organic acids
3-Aminopropanoic acid	1185	amino acids
Malonic acid	1197	organic acids
Acetoacetic acid	1199	fatty acids
3-Aminoisobutyric acid	1200	amino acids
3-Hydroxyisovaleric acid	1200	organic acids
Valine	1204	amino acids
Methylmalonic acid	1205	organic acids
Glyceraldehyde	1207	carbohydrates
methyl serine	1215	amino acids
Dihydroxyacetone	1217	carbohydrates
2-Hydroxyisocaproic acid	1219	organic acids
Urea	1239	amides
Serine	1250	amino acids
oxamic acid	1253	organic acids

Benzoic acid	1255	organic acids
Glycerol	1256	carbohydrates
Leucine	1260	amino acids
Octanoic acid	1260	fatty acids
Phosphoric acid	1260	phosphoric acids
Ethylmalonic acid	1269	organic acids
diethanolamine	1273	alcohols
Isoleucine	1281	amino acids
Acetylglycine	1288	amino acids
threoninol	1290	amino acids
Proline	1291	amino acids
4-Aminobutyric acid	1295	amino acids
Maleic acid	1298	organic acids
Nicotinic acid	1303	organic acids
Phenylacetic acid	1304	organic acids
Norleucine	1307	amino acids
Succinic acid	1307	organic acids
Glyceric acid	1312	carbohydrates
Catechol	1314	phenols
Methylsuccinic acid	1316	organic acids
3-Hydroxypyruvic acid	1322	organic acids
oxamide	1326	amino acids
Uracil	1334	pyrimidines
Fumaric acid	1344	organic acids
Homoserine	1351	amino acids
Nonanoic acid	1357	fatty acids
pipecolic acid	1359	carboxylic acid
2-Propyl-3-hydroxy-pentanoic acid	1365	organic acids
Threonine	1367	amino acids
O-Acetylserine	1383	amino acids
Mevalonic lactone	1384	esters
2-Aminooctanoic acid	1390	amino acids
Mesaconic acid	1390	organic acids
5-Aminovaleric acid	1392	fatty acids
Thymine	1394	pyrimidines
Isobutyrylglycine	1395	amino acids
Glutaric acid	1397	organic acids
Hydroquinone	1397	phenols
3-Methylglutaric acid	1415	organic acids
methyl cysteine	1415	amino acids
Erythorse	1429	carbohydrates
Erythrulose	1444	carbohydrates
Glutaconic acid	1444	organic acids
N-Butyrylglycine	1449	amino acids
Decanoic acid	1453	fatty acids
Oxalacetic acid	1453	organic acids

Citramalic acid	1456	organic acids
2-Propyl-5-hydroxy-pentanoic acid	1465	organic acids
Threitol	1471	carbohydrates
Dihydrouracil	1472	pyrimidines
Malic acid	1472	organic acids
meso-Erythritol	1480	carbohydrates
Isovalerylglycine	1493	amino acids
Adipic acid	1498	organic acids
Niacinamide	1498	pyridines
N-Acetylserine	1499	amino acids
Glutamic acid 5-methylester	1501	amino acids
Anthranilic acid	1502	amino acids
Aspartic acid	1503	amino acids
3-Aminoglutaric acid	1504	organic acids
Salicylic acid	1508	carboxylic acid
4-Hydroxyproline	1511	amino acids
homoglutamine	1511	amino acids
Methionine	1513	amino acids
5-Oxoproline	1522	organic acids
pyroglutamic acid	1523	amino acids
Cytosine	1525	pyrimidines
3-Methyladipic acid	1527	organic acids
Thiodiglycolic acid	1529	organic acids
Threonic acid	1530	carbohydrates
Pyrogallol	1531	phenols
Phenylpyruvic acid	1532	organic acids
2-Propyl-glutaric acid	1535	organic acids
Cysteine	1543	amino acids
Tiglylglycine	1545	amino acids
5-Hydroxymethyl-2-furoic acid	1547	organic acids
Creatinine	1548	amides
2-Hydroxyglutaric acid	1556	organic acids
3-Hydroxyglutaric acid	1557	organic acids
Succinylacetone	1559	organic acids
2-Isopropylmalic acid	1560	organic acids
3-Methylcrotonoylglycine	1560	amino acids
cinnamic acid	1563	carboxylic acid
O-Phosphoethanolamine	1565	esters
2-Ketoglutaric acid	1566	organic acids
3-Phenyllactic acid	1579	organic acids
3-Hydroxy-3-methylglutaric acid	1584	organic acids
Phosphoenolpyruvic acid	1584	organic acids
Tropic acid	1584	organic acids
Hypotaurine	1595	organic acids
Pimelic acid	1595	organic acids
Ornithine	1597	amino acids

Asparagine	1602	amino acids
Triethanolamine	1604	alcohols
Cadaverine	1605	amines
3-Hydroxyphenylacetic acid	1606	organic acids
Tartaric acid	1611	organic acids
Lyxose	1619	carbohydrates
2-Ketoadipic acid	1622	organic acids
Phenylalanine	1623	amino acids
Ureidopropionic acid	1624	amino acids
Xylose	1626	carbohydrates
4-Hydroxybenzoic acid	1627	organic acids
Arabinose	1632	carbohydrates
Threo-b-hydroxyaspartic acid	1633	amino acids
Hexanoylglycine	1636	organic acids
4-Hydroxyphenylacetic acid	1638	organic acids
Xylulose	1642	carbohydrates
lauric acid	1646	fatty acids
Ribulose	1646	carbohydrates
Ribose	1648	carbohydrates
N-Acetylaspartic acid	1650	amino acids
Homocysteine	1653	amino acids
Taurine	1663	organic acids
3-Sulfinoalanine	1668	amino acids
Ribonolactone	1668	carbohydrates
Xylitol	1672	carbohydrates
Arabitol	1685	carbohydrates
Rhamnose	1686	carbohydrates
Glyceraldehyde 3-phosphate	1688	carbohydrates
Lysine	1689	amino acids
Ribitol	1689	carbohydrates
1,6-Anhydroglucose	1691	carbohydrates
Suberic acid	1691	organic acids
Glycerol 2-phosphate	1695	carbohydrates
Fucose	1697	carbohydrates
2-Aminoadipic acid	1698	amino acids
2-Deoxy-glucose	1698	carbohydrates
Glutamine	1702	amino acids
Cysteic acid	1723	amino acids
Putrescine	1723	amines
Ribonic acid	1728	organic acids
Quinolinic acid	1730	pyridines
Aconitic acid	1732	organic acids
Orotic acid	1732	organic acids
Glycerol 3-phosphate	1734	carbohydrates
Dihydroxyacetone phosphate	1736	carbohydrates
tridecanoic acid	1744	fatty acids

5-Aminolevulinic acid	1748	amino acids
2-Phosphoglyceric acid	1753	organic acids
tetradecanol	1755	fatty alcohol
3-Hydroxyanthranilic acid	1761	amino acids
3-Methoxy-4-hydroxybenzoic acid	1764	organic acids
Dihydroorotic acid	1765	organic acids
3-Dehydroshikimic acid	1768	organic acids
Homovanillic acid	1769	organic acids
3-Phosphoglyceric acid	1781	organic acids
Shikimic acid	1783	organic acids
Azelaic acid	1788	organic acids
Glycyl-Glycine	1793	amides
Isocitric acid	1793	organic acids
2-Aminopimelic acid	1794	amino acids
Citric acid	1794	organic acids
Hippuric acid	1805	organic acids
Arginine	1808	amino acids
Protocatechuic acid	1808	organic acids
Tagatose	1812	carbohydrates
O-Phospho-Serine	1814	amino acids
Dopamine	1815	phenols
Hypoxanthine	1816	purines
Psicose	1819	carbohydrates
quinic acid	1819	carboxylic acid
Homogentisic acid	1821	organic acids
Epinephrine	1829	phenols
1,5-Anhydro-glucitol	1831	carbohydrates
Methionine sulfone	1833	amino acids
myristoleic acid	1833	fatty acids
Sorbose	1833	carbohydrates
2-Methylhippuric acid	1834	organic acids
Fructose	1834	carbohydrates
Pyridoxal	1835	pyridines
Histidinol	1842	alcohols
Allose	1843	carbohydrates
Galactosamine	1843	carbohydrates
Myristic acid	1844	fatty acids
Mannose	1849	carbohydrates
5-Dehydroquinic acid	1858	organic acids
Vanilmandelic acid	1859	organic acids
Glucono-1,5-lactone	1860	carbohydrates
Allantoin	1868	amides
gluconic acid lactone	1870	polyhydroxy acid
Vanillylamine	1870	amines
Histamine	1876	amines
N6-Acetyllysine	1878	amino acids

Erythrose 4-phosphate	1879	carbohydrates
Galactose	1879	carbohydrates
Adenine	1881	purines
Glucose	1882	carbohydrates
N-Acetyl-Lysine	1882	amino acids
Glucosamine	1884	carbohydrates
Pyridoxine	1884	pyridines
Mannitol	1885	carbohydrates
Sebacic acid	1885	organic acids
Galactitol	1898	carbohydrates
N-Acetylglutamine	1898	amino acids
Sorbitol	1898	carbohydrates
4-Hydroxyphenylpyruvic acid	1901	organic acids
Glucuronic acid	1902	carbohydrates
Glucono-1,4-lactone	1903	carbohydrates
Galacturonic acid	1909	carbohydrates
Tyramine	1909	phenols
Histidine	1912	amino acids
3,4-Dihydroxybenzylamine	1917	amines
Urocanic acid	1919	organic acids
Ureidosuccinic acid	1924	organic acids
Ascorbic acid	1926	carbohydrates
saccharo-1,4-lactone	1927	carbohydrates
Pyridoxamine	1929	pyridines
Tyrosine	1929	amino acids
cis-10-pentadecenoic acid	1935	fatty acids
2-Hydroxyhippuric acid	1940	organic acids
Coniferyl alcohol	1940	phenols
pentadecanoic acid	1941	fatty acids
coumaric acid	1944	carboxylic acid
1-Hexadecanol	1951	alcohols
Indol-3-acetic acid	1951	Indoles
N-Acetyl-Ornithine	1952	amino acids
Coniferyl aldehyde	1958	phenols
Gluconic acid	1963	carbohydrates
Tryptamine	1972	Indoles
Pantothenic acid	1974	amides
Glucaric acid	1977	carbohydrates
Lipoic acid	1977	fatty acids
ParaXanthine	1993	purines
S-Benzyl-Cysteine	2000	amino acids
Octopamine	2008	alcohols
Xanthine	2016	purines
Palmitoleic acid	2026	fatty acids
Palmitic acid	2040	fatty acids
N-Acetylmannosamine	2049	carbohydrates

Inositol	2058	carbohydrates
Ribulose 5-phosphate	2062	carbohydrates
Ribose 5-phosphate	2066	carbohydrates
Dopa	2075	amino acids
Dodecanedioic acid	2078	organic acids
Kynurenic acid	2080	organic acids
Uric acid	2082	purines
Metoprolol	2084	alcohols
ferulic acid	2095	phenolic acid
Citrulline	2100	amino acids
cis-10-heptadecenoic acid	2123	fatty acids
Methoprene acid	2126	terpenoids
Guanine	2127	purines
Norepinephrine	2130	phenols
N-Acetyltyrosine	2131	amino acids
heptadecanoic acid	2137	fatty acids
Margaric acid	2138	fatty acids
Kynurenine	2139	amino acids
Octadecanol	2147	alcohols
2,3-Bisphosphoglyceric acid	2160	carbohydrates
Cystathionine	2189	amino acids
Cystamine	2199	amines
7-Methylguanine	2200	purines
Linoleic acid	2210	fatty acids
pyrrolnitrin	2213	pyrroles
Oleic acid	2214	fatty acids
Tryptophan	2214	amino acids
Suberylglycine	2215	amino acids
alpha-linolenic acid	2217	fatty acids
Elaidic acid	2221	fatty acids
Spermidine	2230	amines
Stearic acid	2238	fatty acids
Fructose 1-phosphate	2244	carbohydrates
sinapic acid	2245	carboxylic acid
Fructose 6-phosphate	2259	carbohydrates
Mannose 6-phosphate	2260	carbohydrates
Glucose 6-phosphate	2274	carbohydrates
Cystine	2276	amino acids
Methyl arachidonate	2277	fatty acids
norhydrocapsiate	2292	fatty acids
nonadecanoic acid	2334	fatty acids
eicosanol	2340	alcohols
3-Hydroxy-kynurenine	2347	amino acids
2'-Deoxyuridine	2371	nucleotides
6-Phosphogluconic acid	2374	carbohydrates
Juniperic acid	2377	fatty acids

Eicosapentaenoic acid	2380	fatty acids
8, 11, 14-eicosatrienoic acid	2390	fatty acids
dihydrocapsiate	2391	fatty acids
Porphobilinogen	2404	organic acids
11-eicosaenoic acid	2411	fatty acids
Inositol phosphate	2412	carbohydrates
11-eicosenoic acid	2415	fatty acids
Thymidine	2416	nucleotides
5-Hydroxy-tryptophan	2420	amino acids
Oleamide	2421	amides
Uridine	2430	nucleotides
arachidic acid	2432	fatty acids
5-Methoxytryptamine	2439	Indoles
Saccharopine	2440	amino acids
p-Aminohippuric acid	2460	amino acids
Biotin	2480	organic acids
Melatonin	2485	Indoles
Sedoheptulose 7-phosphate	2496	carbohydrates
Homocystine	2506	amino acids
heneicosanoic acid	2531	fatty acids
docosanol	2536	alcohols
Inosine	2555	purines
Docosahexaenoic acid	2565	fatty acids
Sucrose	2579	fatty acids
N-Acetylneuraminic acid	2580	carbohydrates
Docosapentaenoic acid	2581	fatty acids
lactulose	2620	carbohydrates
Adenosine	2621	purines
Lactose	2621	carbohydrates
cellobiose	2626	carbohydrates
behenic acid	2630	fatty acids
Fendiline	2636	amines
Xanthosine	2644	nucleostdes
Batyl alcohol	2651	alcohols
Turanose	2675	carbohydrates
Maltose	2678	carbohydrates
Trehalose	2683	carbohydrates
Cytidine	2684	nucleotides
Lactitol	2699	carbohydrates
Spermine	2713	amines
tricosanoic acid	2729	fatty acids
tetracosanol	2732	fatty acids
Carnosine	2737	amides
Guanosine	2743	purines
Monostearin	2759	glycerides
Maltitol	2768	carbohydrates

5'-Methylthioadenosine	2772	nucleotides
palatinose	2774	carbohydrates
Isomaltose	2804	carbohydrates
melibiose	2820	carbohydrates
Uridine monophosphate	2824	nucleotides
tetracosanoic acid	2831	fatty acids
Thymidine monophosphate	2866	nucleotides
delta-tocopherol	2902	vitamin
Benzylpalmitamide	2913	fatty amide
hexacosanol	2928	fatty alcohol
Inosine monophosphate	2964	purines
Xanthosine monophosphate	3024	nucleotides
Cholecalciferol	3038	steroids
Adenosine monophosphate	3043	nucleotides
Trehalose 6-phosphate	3111	carbohydrates
Adenosine 3',5'-cyclic monophosphate	3113	nucleotides
octacosanol	3123	fatty alcohol
alpha-tocopherol	3154	vitamin
Cholesterol	3198	steroids
Cholestanol	3208	steroids
raffinose	3280	carbohydrates
campesterol	3283	steroids
Stigmasterol	3306	steroids
Triacontanol	3330	fatty alcohol
Sitosterol	3375	steroids
lanosterol	3383	triterpenoid
maltotriose	3462	carbohydrates

Tabe 4. The classifications of the 394 compounds in Metabolomics method

Classification	Number	Classification	Number
Alcohols	12	Organic acid	94
Amides	7	Phenols	9
Amines	9	Phenolic acid	1
Amino acids	77	Phosphoric acid	1
Carbohydrates	77	Polyhydroxy acid	1
Caboxylic acids	6	Purines	11
Esters	2	Pyridines	7
Fatty acids	42	Pyrimidines	4
Fatty alcohol;s	4	Pyrrole	1
Glyceride	1	Steroids	6
Indoles	4	Terpenoid	1
Inorganic compound	1	Triterpenoid	1
Nucleotides	11	Vitamins	2

Trimethylsilylation of methoximated compounds

In the case of pyruvate, malic acid, glucose, palmitate or similar metabolites, GC-MS requires a derivatization step that makes these compounds sufficiently volatile for analysis. The most common derivatization protocol uses trimethylsilylation (Kumari et al, 2011), (Fiehn et al, 2010) to remove acidic protons from hydroxyl-, carboxyl-, amino- or thiol groups. These derivatization reactions work quickly under very mild conditions, break the molecular proton bonds with very high yields, lower the boiling point for GC-MS analysis and increase the stability of the compound (Laine & Sweeley, 1971).

Figure 3. Trimethylsilylation of methoximated glucose

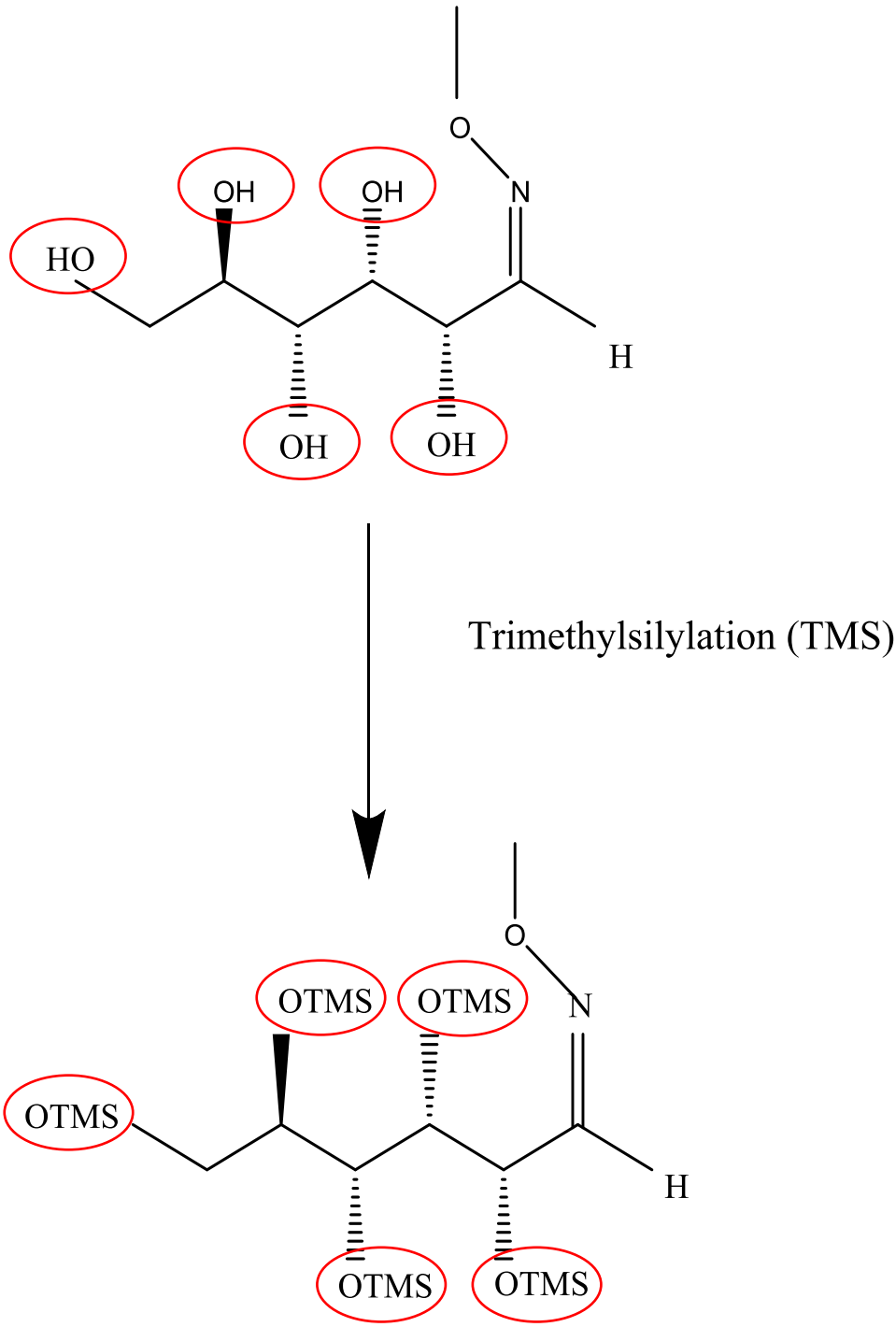
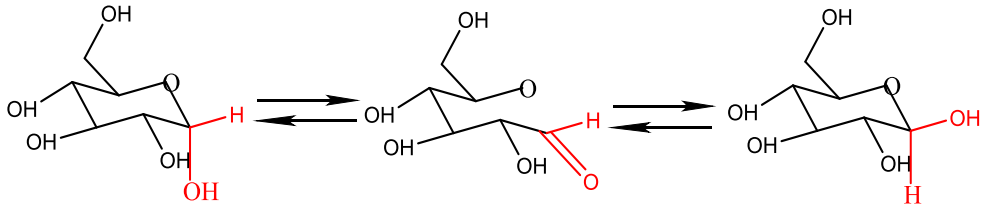

O
N
OH
OH
HO
H
OH
OH
Trimethylsilylation (TMS)
O
N
OTMS
OTMS
OTMS
H
OTMS
OTMS

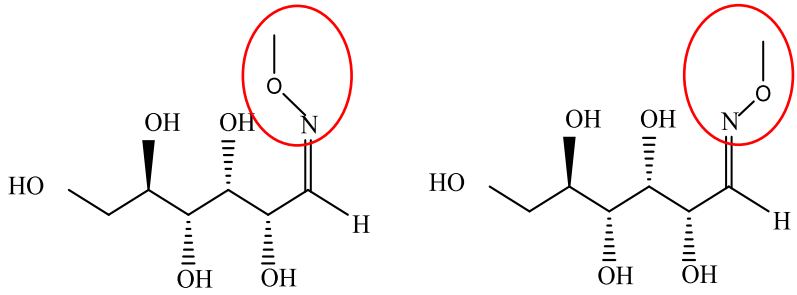
Figure 4. Methoximation of glucose



α-glucose

β-glucose

Methoximized



Results

Figure 5. one-way ANOVA with 0,05P-value Miyamoto, Hanlabong Hwangkeumhyang, Redhyang flesh

Adjusted p-value (FDR) cutoff: 0.05

Post-hoc analysis: Fisher\'s LSD

Non-parametric ANOVA:

Submit

Click on a point to view, drag to zoom

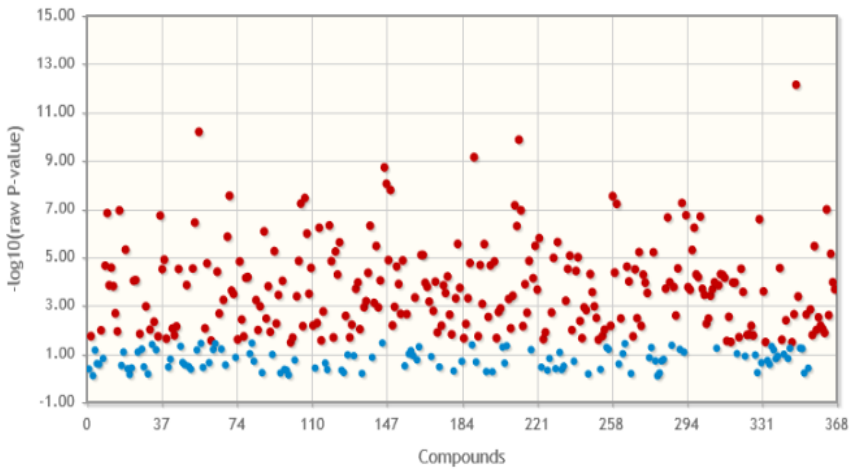


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Figure 6. PCA score plot of Miyamoto, Hanlabong, Hwangkeumhyang,, Redhyang flesh

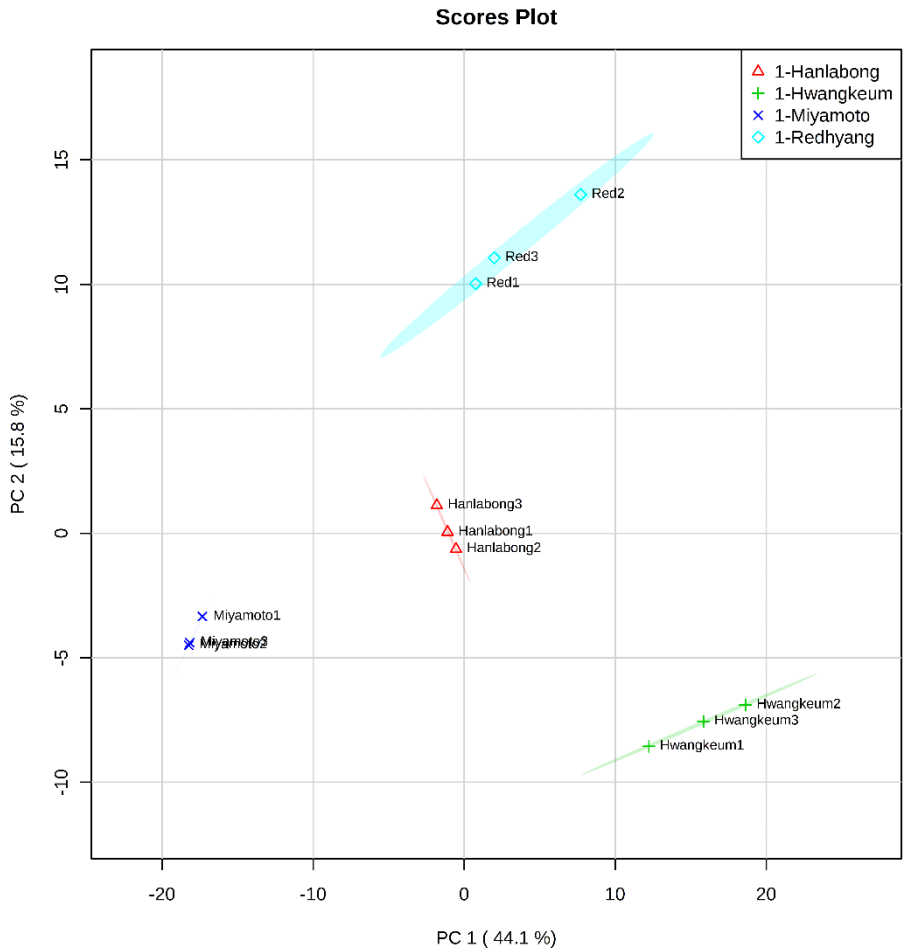
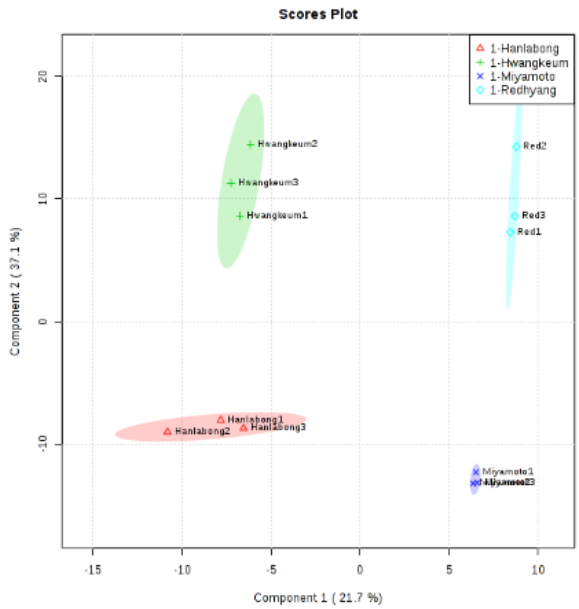

Scores Plot
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang
Red2
Red3
Red1
Hanlabong3
Hanlabong1
Hanlabong2
Miyamoto1
Hwangkeum2
Hwangkeum3
Hwangkeum1
PC 2 (15.8 %)
PC 1 (44.1 %)
15
10
5
0
-5
-10
-20
-10
0
10
20

Figure 7. (A) PLS-DA score plot of Miyamoto, Hanlabong, Hwangkeumhyang, Redhyang flesh

(B) PLS-DA VIP score of Miyamoto, Hanlabong, Hwangkeum-hyang, Redhyang flesh

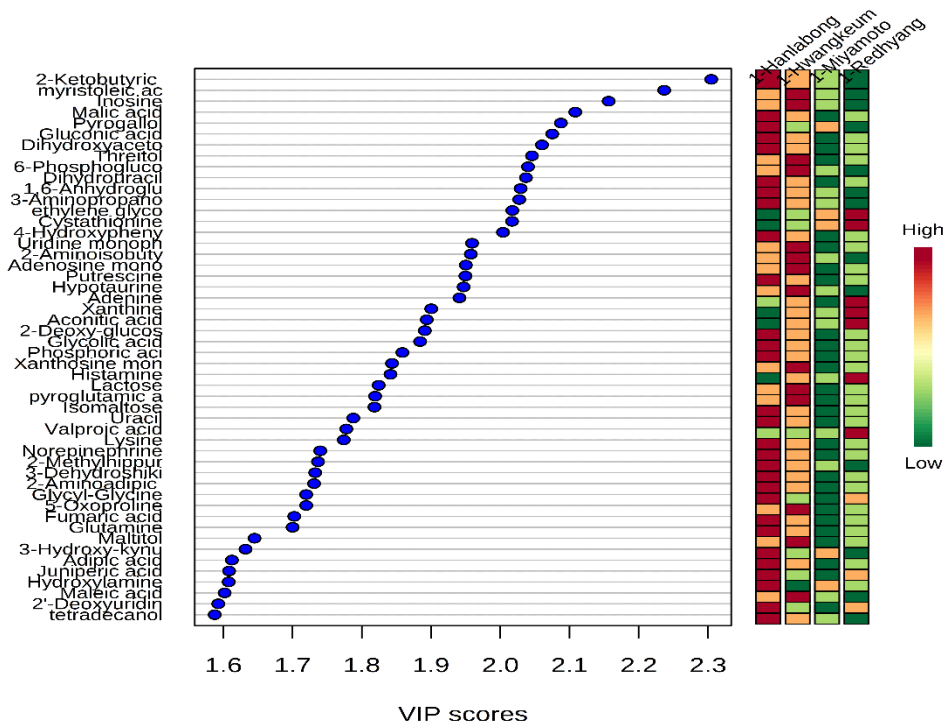
(C) Heatmap of Miyamoto, Hanlabong, Hwangkeumhyang, Redhyang flesh

(A)



Note, PLS-DA maximizes the **covariance** between X (data) and Y (group). The variance displayed in the plot above is the **explained variance for X**. Covariance and x-variance may not agree with each other in some cases. For instance, the 1st component may not explain more X-variance than the 2nd component.

(B)



(C)

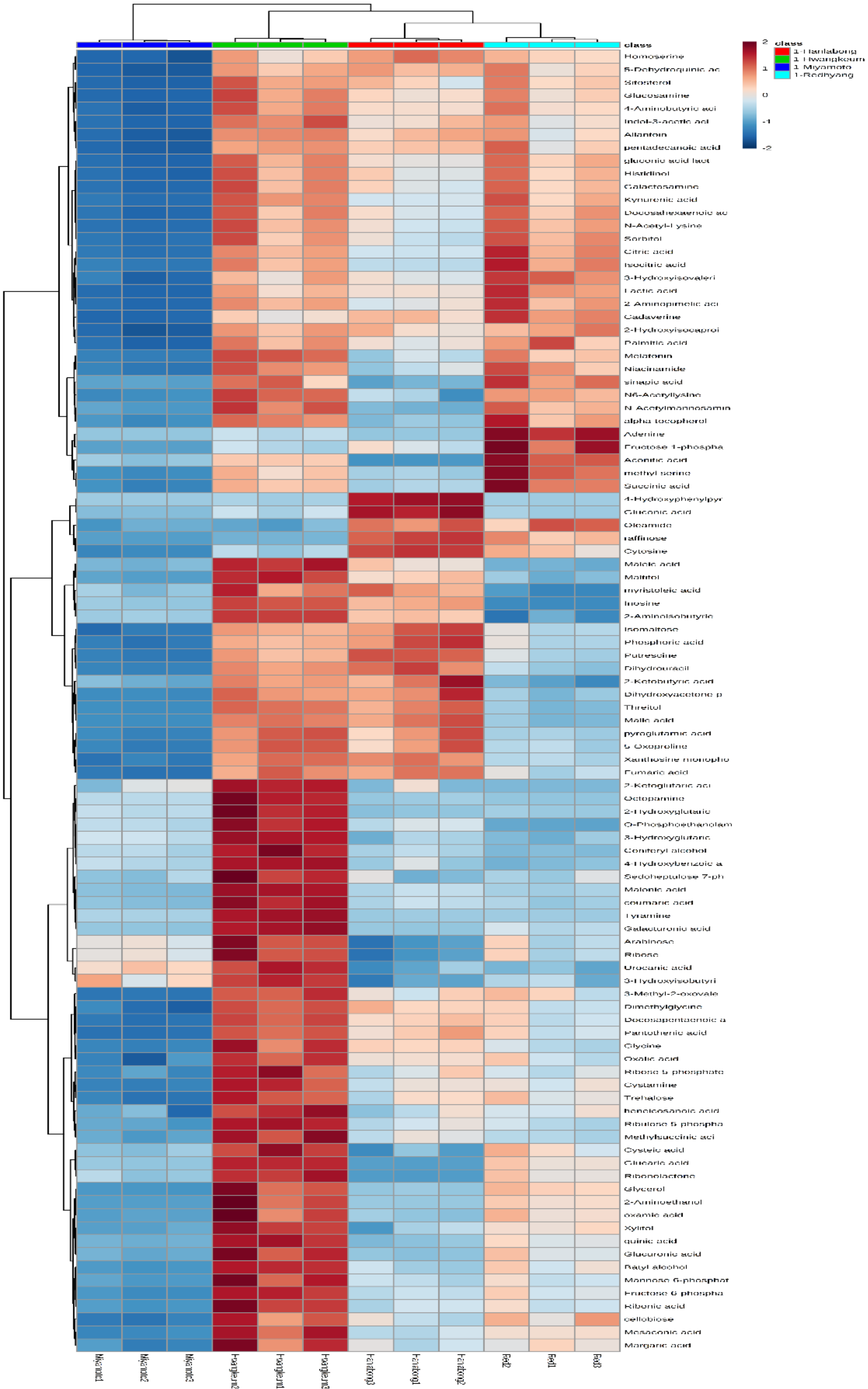
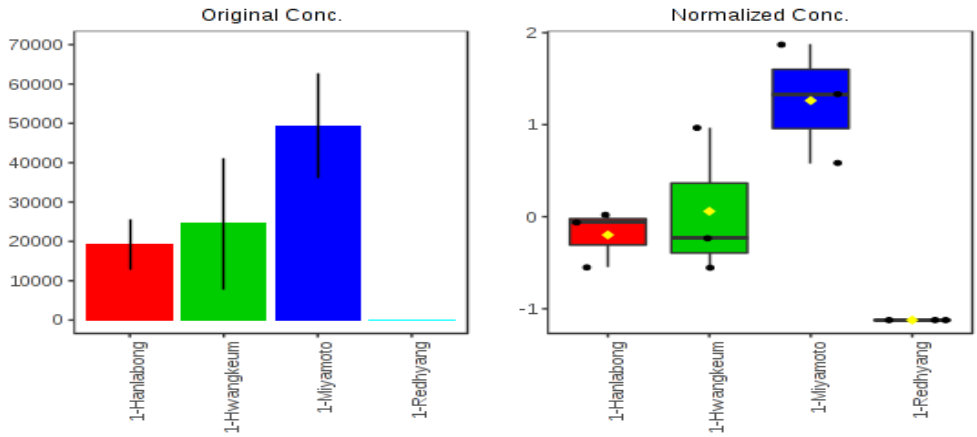
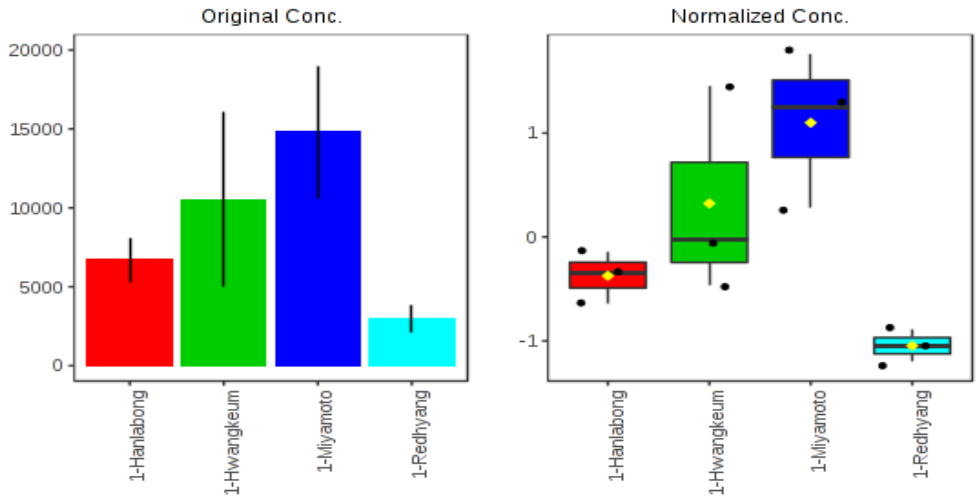

class
Homoserine
5-Dehydroquinic ac
Sitosterol
Glucosamine
4-Aminobutyric aci
Indol-3-acetic aci
Allantoin
pentadecanoic acid
gluconic acid lact
Histidinol
Galactosamine
Kynurenic acid
Docosahexaenoic ac
N-Acetyl-Lysine
Sorbitol
Citric acid
Isocitric acid
3-Hydroxyisovaleri
Lactic acid
2 Aminopimelic aci
Cadaverine
2-Hydroxyisocaproi
Palmitic acid
Melatonin
Niacinamide
sinapic acid
N6-Acetyllysine
N Acetylmannosamin
alpha tocopherol
Adenine
Fructose 1-phospha
Aconitic acid
methyl serine
Succinic acid
4-Hydroxyphenylpyr
Gluconic acid
Oleamide
raffinose
Cytosine
Maleic acid
Maltitol
myristoleic acid
Inosine
2-Aminoisobutyric
Isomaltose
Phosphoric acid
Putrescine
Dihydrouracil
2-Ketobutyric acid
Dihydroxyacetone p
Threitol
Malic acid
pyroglutamic acid
5 Oxoproline
Xanthosine monopho
Fumaric acid
2-Ketoglutaric aci
Octopamine
2-Hydroxyglutaric
O-Phosphoethanolam
3-Hydroxyglutaric
Coniferyl alcohol
4-Hydroxybenzoic a
Sedoheptulose 7-ph
Malonic acid
coumaric acid
Tyramine
Galacturonic acid
Arabinose
Ribose
Urocanic acid
3-Hydroxyisobutyri
3-Methyl-2-oxovale
Dimethylglycine
Docosapentaenoic a
Pantothenic acid
Glycine
Oxalic acid
Ribose 5 phosphate
Cystamine
Trehalose
heneicosanoic acid
Ribulose 5 phospha
Methylsuccinic aci
Cysteic acid
Glucaric acid
Ribonolactone
Glycerol
2-Aminoethanol
oxamic acid
Xylitol
quinic acid
Glucuronic acid
Batyl alcohol
Mannose 6-phosphat
Fructose 6 phospha
Ribonic acid
cellobiose
Mesaconic acid
Margaric acid
2
1
0
-1
-2
class
1-Hanlabong
1 Hwangkeum
1 Miyamoto
1-Redhyang

Figure 8. compound to specify Miyamoto flesh

N-Acetylneuraminic acid



Inositol



Glucono-1,5-lactone

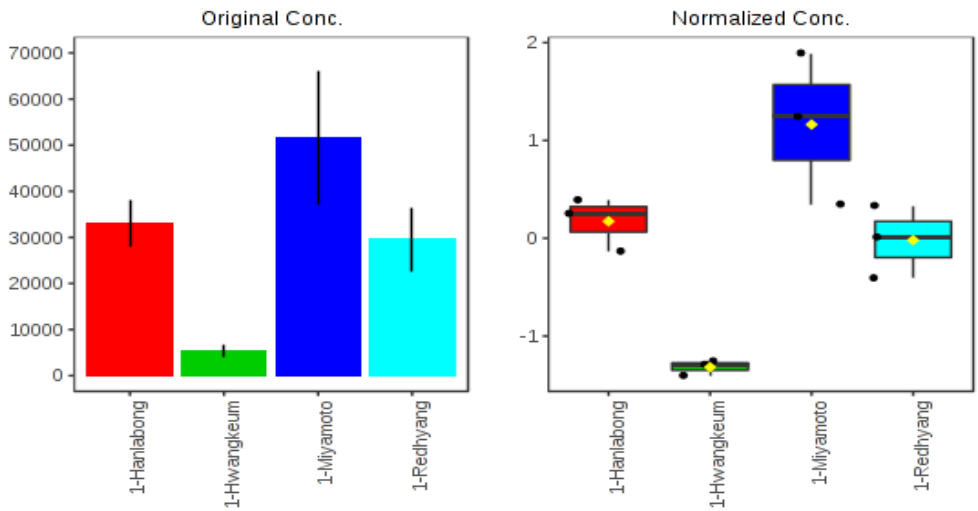
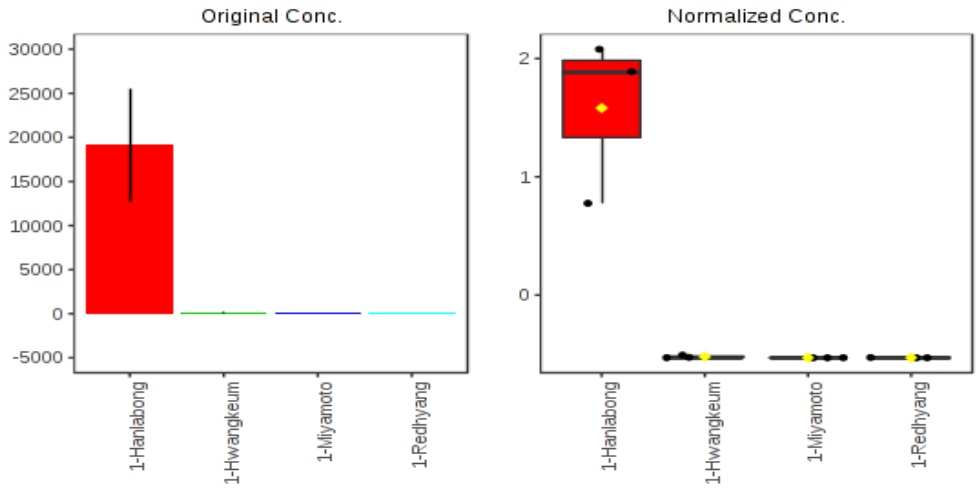
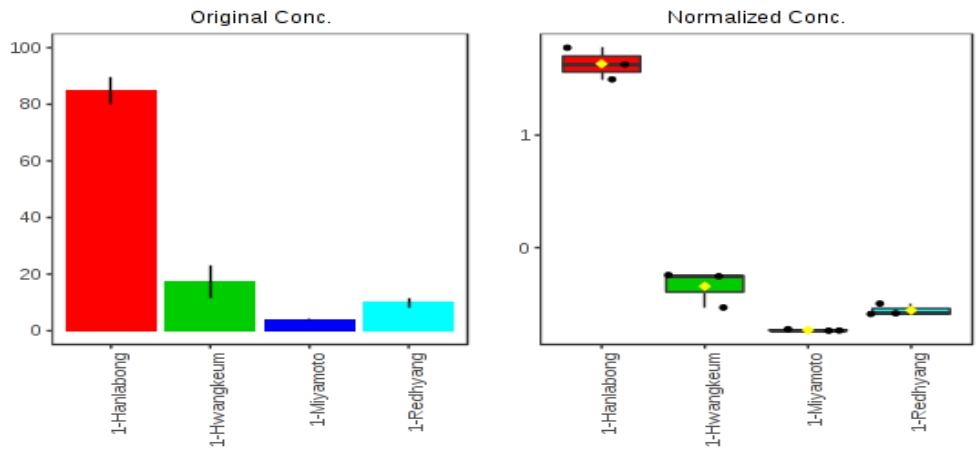


Figure 9. compound to specify Hanlabong flesh

2-Deoxy-glucose



Gluconic acid



4-Hydroxyphenylpyruvic acid

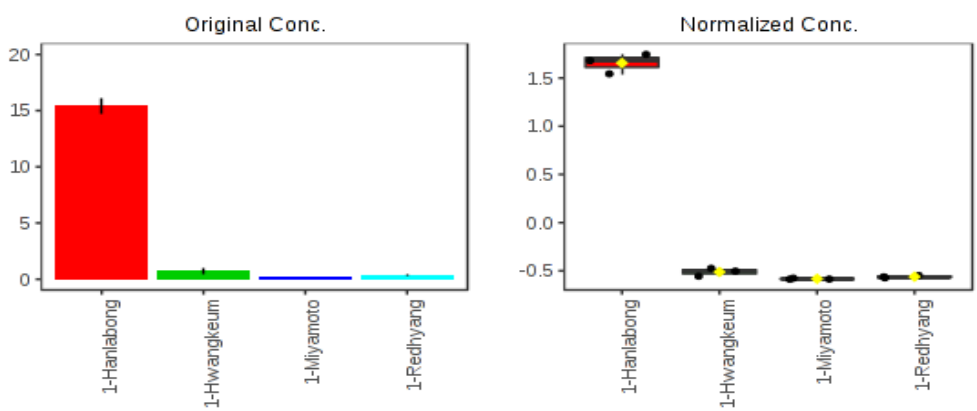
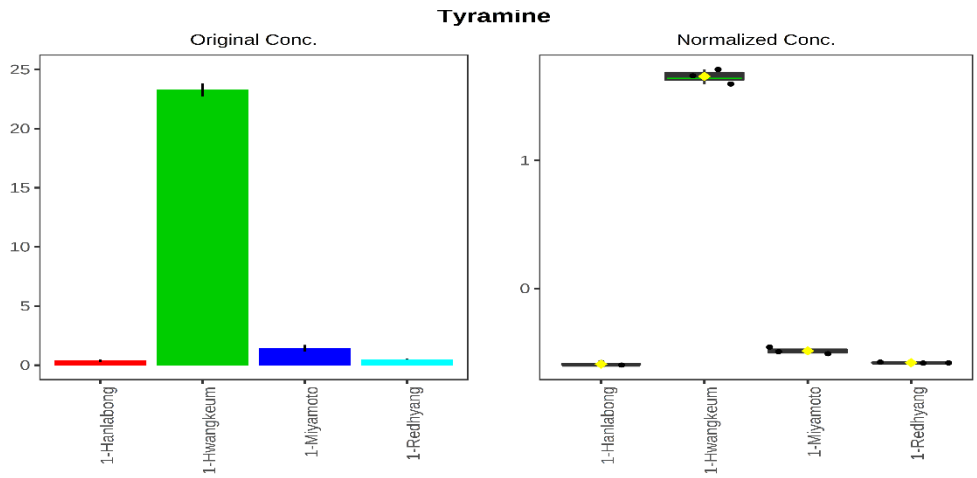
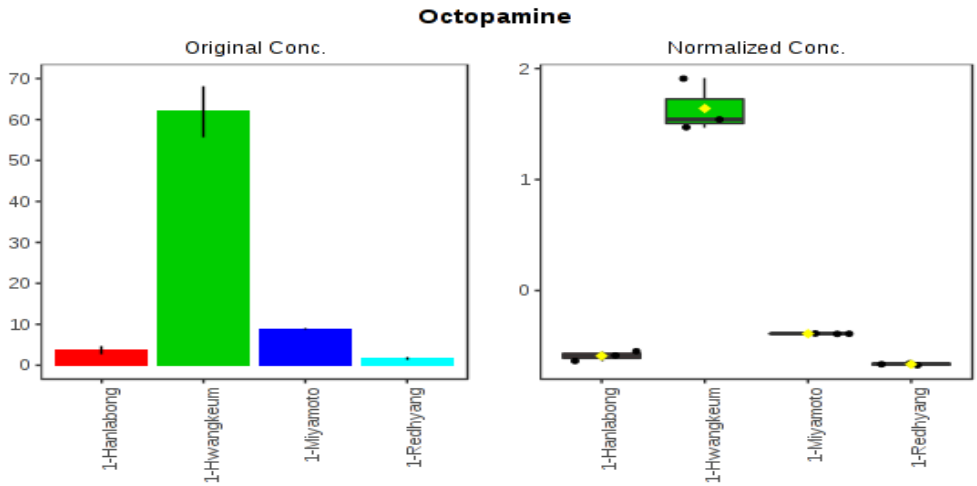


Figure 10. compound to specify Hwangkeumhyang flesh


Tyramine
Original Conc.
Normalized Conc.
25
20
15
10
5
0
1
0
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang


Octopamine
Original Conc.
Normalized Conc.
70
60
50
40
30
20
10
0
2
1
0
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang

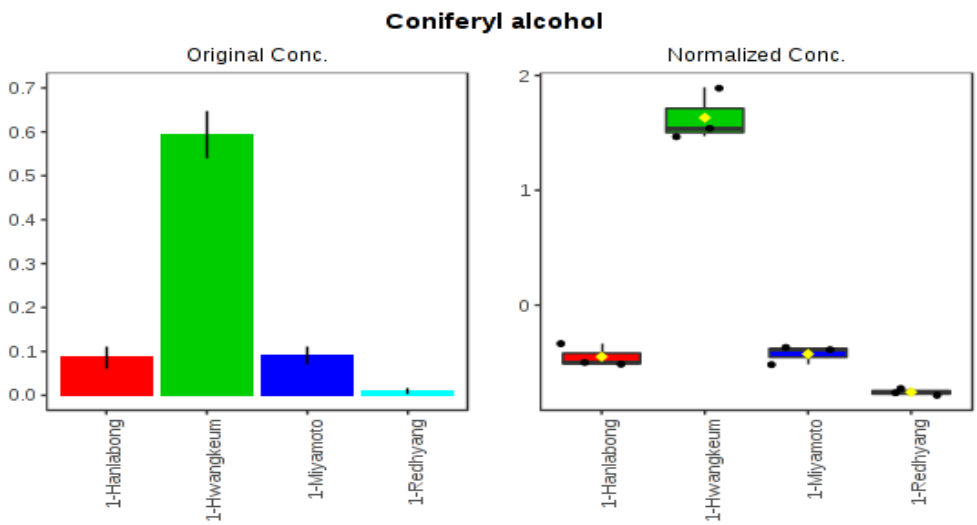
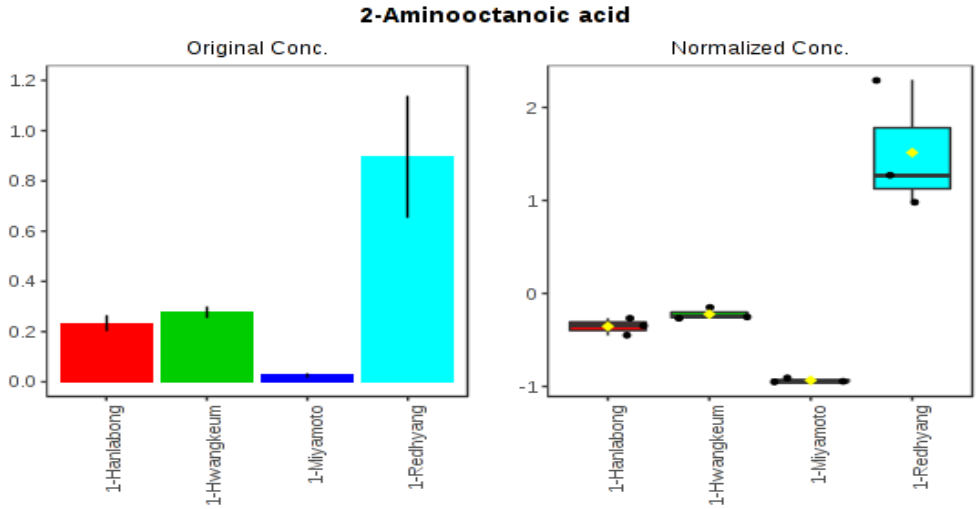
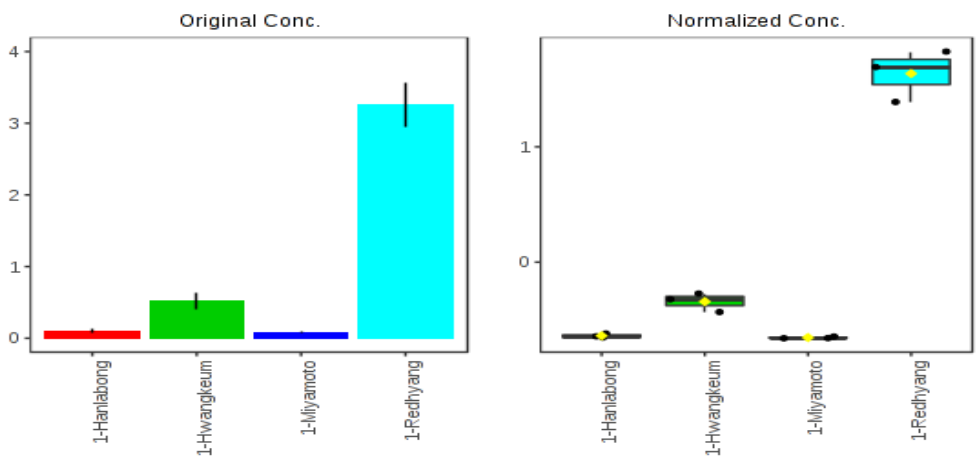

Coniferyl alcohol
Original Conc.
Normalized Conc.
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
2
1
0
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang

Figure 11. compound to specify Redhyang flesh


2-Aminooctanoic acid
Original Conc.
Normalized Conc.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2
1
0
-1
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang


Adenine
Original Conc.
Normalized Conc.
4
3
2
1
0
1
0
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang

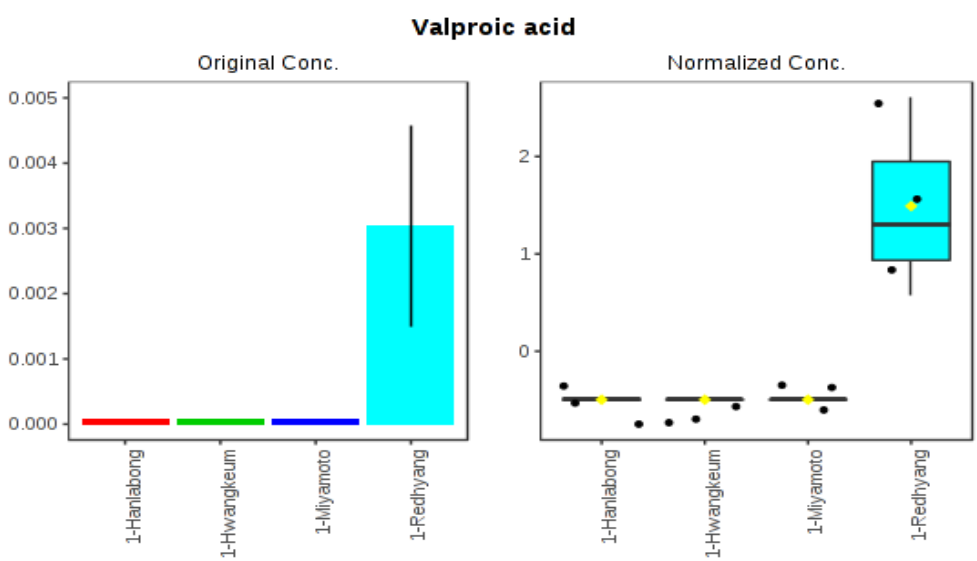

Valproic acid
Original Conc.
Normalized Conc.
0.005
0.004
0.003
0.002
0.001
0.000
2
1
0
1-Hanlabong
1-Hwangkeum
1-Miyamoto
1-Redhyang

Figure 12. one-way ANOVA with 0,05P-value Miyamoto, Hanlabong Hwangkeumhyang, Redhyang peel

Adjusted p-value (FDR) cutoff: 0.05

Post-hoc analysis: Fisher\'s LSD

Non-parametric ANOVA:

Submit

Click on a point to view, drag to zoom

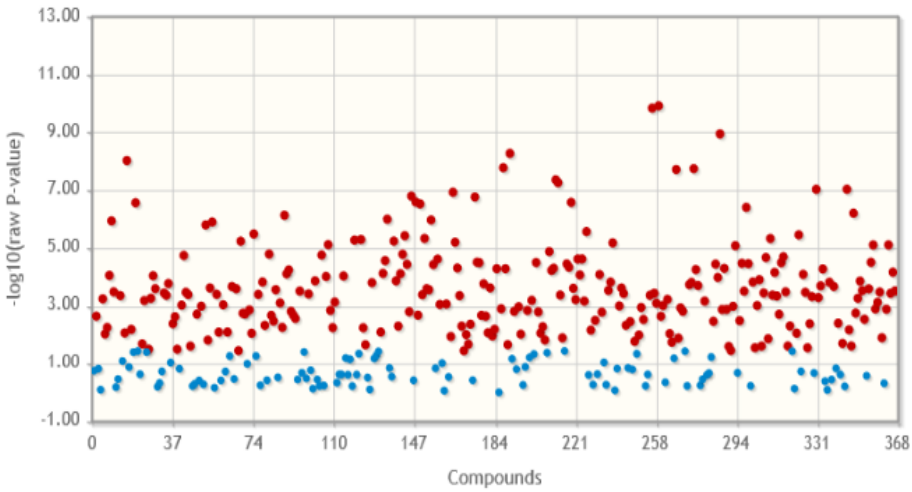


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Figure 13. PCA score plot of Miyamoto, Hanlabong, Hwangkeumhyang,, Redhyang peel

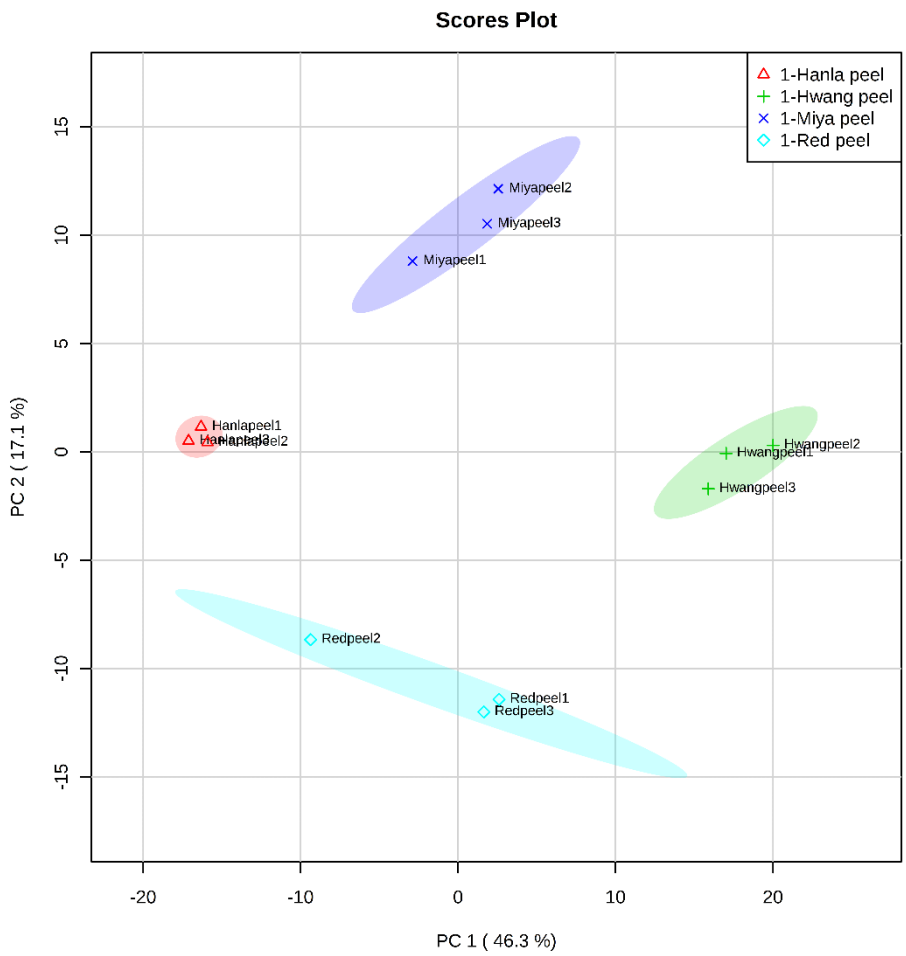
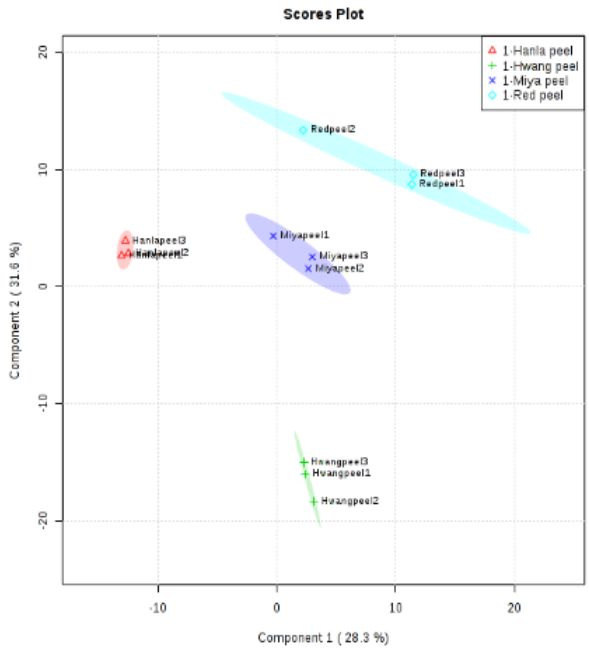

Scores Plot
1-Hanla peel
1-Hwang peel
1-Miya peel
1-Red peel
Miyapeel2
Miyapeel3
Miyapeel1
Hanlapeel1
Hwangpeel2
Hwangpeel1
Hwangpeel3
Redpeel2
Redpeel1
Redpeel3
PC 2 (17.1 %)
PC 1 (46.3 %)
15
10
5
0
-5
-10
-15
-20
-10
0
10
20

Figure 14. (A) PLS-DA score plot of Miyamoto, Hanlabong, Hwangkeumhyang, Redhyang peel

(B) PLS-DA VIP score of Miyamoto, Hanlabong, Hwangkeumhyang, Redhyang peel

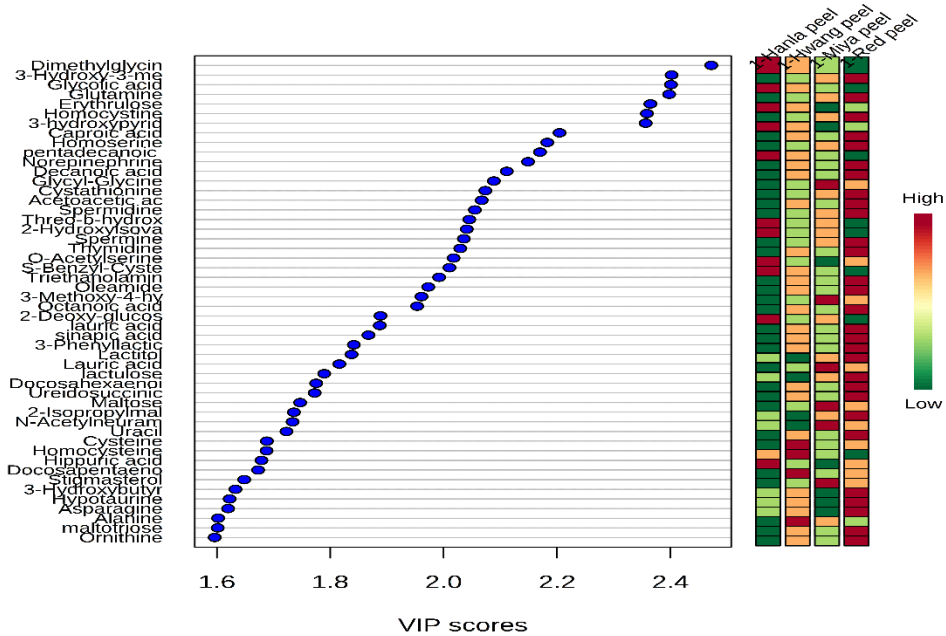
(C) Heatmap of Miyamoto, Hanlabong, Hwangkeumhyang, Redhyang peel

(A)



le, PLS-DA maximizes the **covariance** between X (data) and Y (group). The variance displayed in the plot above is the **explained variance for X**. Covariance and x-variance y not agree with each other in some cases. For instance, the 1st component may not explain more X-variance than the 2nd component.

(B)



(C)

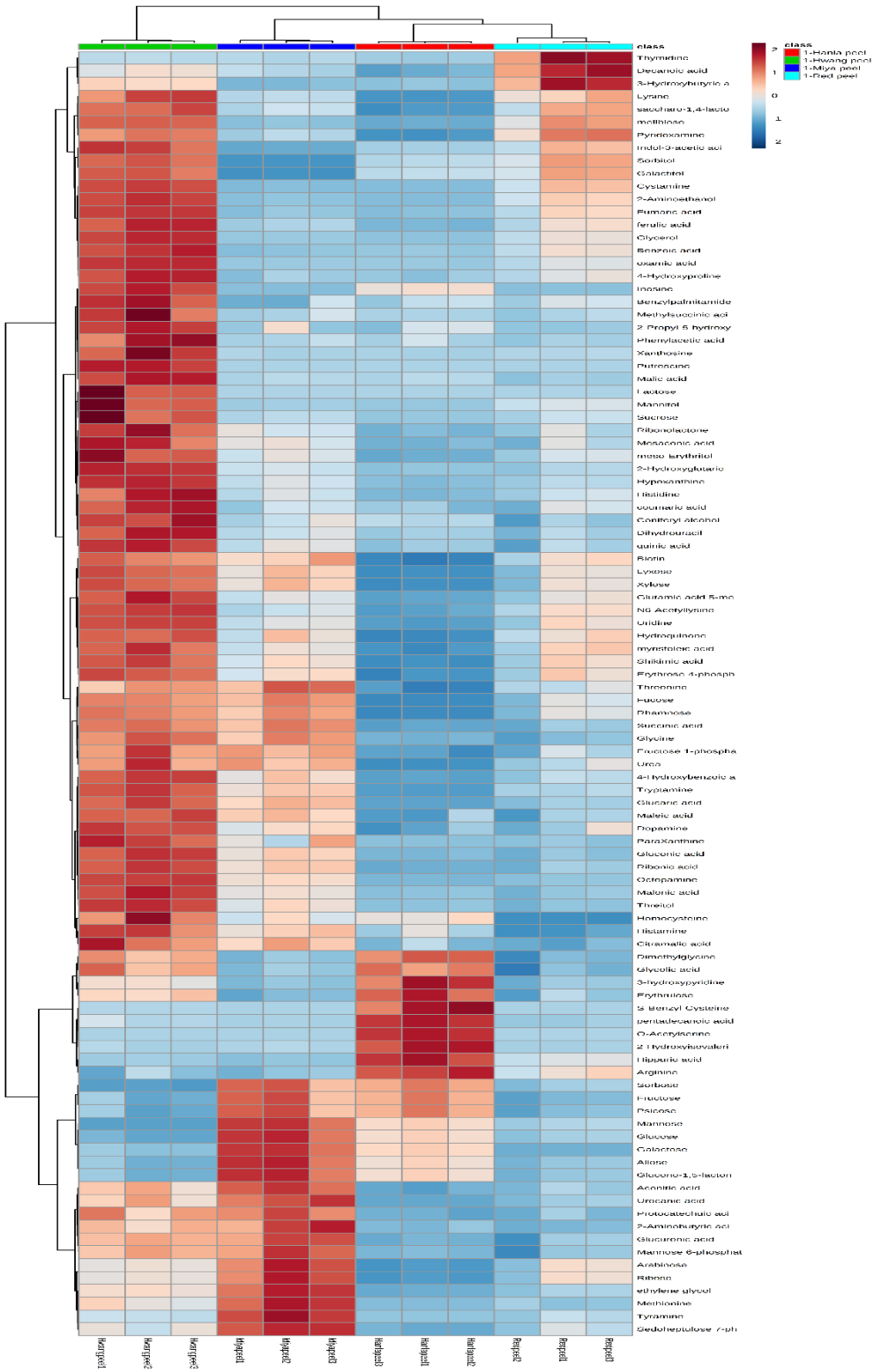
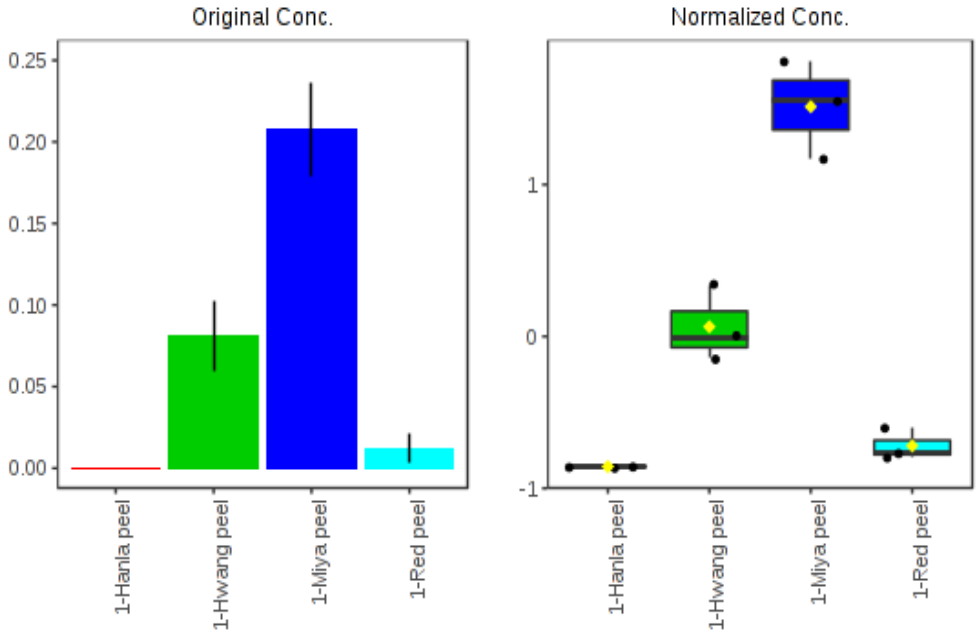

class
1-Hanla peel
1-Hwang peel
1-Miya peel
1-Red peel
Thymidine
Decanoic acid
3-Hydroxybutyric a
Lysine
saccharo-1,4-lacto
maltitose
Pyridoxamine
Indol-3-acetic acl
Sorbitol
Galactitol
Cystamine
2-Aminoethanol
Fumaric acid
ferulic acid
Glycerol
Benzoic acid
oxamic acid
4-Hydroxyproline
Inosine
Benzylpalmitamide
Methylsuccinic acl
2 Propyl 6 hydroxy
Phenylacetic acid
Xanthosine
Putrescine
Malic acid
Lactose
Mannitol
Sucrose
Piperolactone
Mesaconic acid
meso Erythritol
2-Hydroxyglutaric
Hypoxanthine
l Histidine
Coumaric acid
Conitoryl alcohol
Dihydrouracil
quinic acid
Biorin
Lyxose
Xylose
Glutamic acid 5-mo
N6 Acetyllysine
Uridine
Hydroquinone
myristoleic acid
Shikimic acid
Erythrose 4-phosph
Threonine
Fucose
Rhamnose
Succinic acid
Glycine
Fructose 1-phospha
Urea
4-Hydroxybenzoic a
Tryptamine
Glucaric acid
Maleic acid
Dopamine
ParaXanthine
Gluconic acid
Ribonic acid
Octopamine
Malonic acid
Threitol
Homocysteine
l Histamine
Citramalic acid
Dimethylglycine
Glycolic acid
3-hydroxypyridine
Erythrulose
S Benzyl Cysteine
pentadecanoic acid
O-Acetylserine
2 Hydroxyisovaleri
l Hippuric acid
Arginine
Sorbose
Fructose
Psicose
Mannose
Glucose
Galactose
Allose
Gluconic-1,5-lacton
Aconitic acid
Ureidic acid
Protocatechuic acl
2-Aminobutyric acl
Glucuronic acid
Mannose 6-phosphat
Arabinose
Ribose
ethylene glycol
Methionine
Tyramine
Sedoheptulose 7-ph
Hanlipeel1
Hanlipeel2
Hanlipeel3
Miyapeel1
Miyapeel2
Miyapeel3
Hanlipeel1
Hanlipeel2
Hanlipeel3
Redpeel2
Redpeel1
Redpeel3

Figure 15. compound to specify Miyamoto peel

Methionine



Tyramine

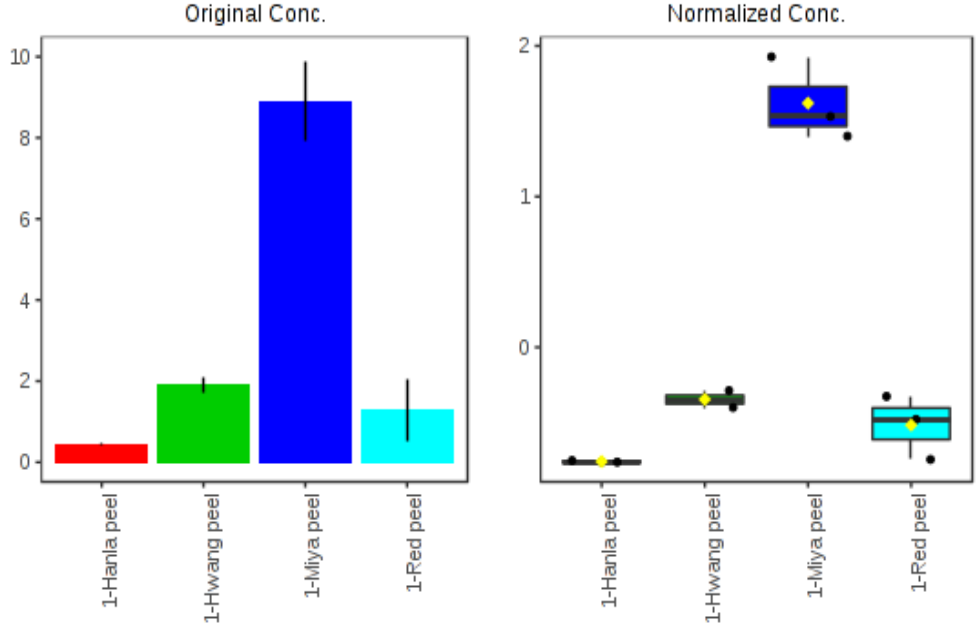
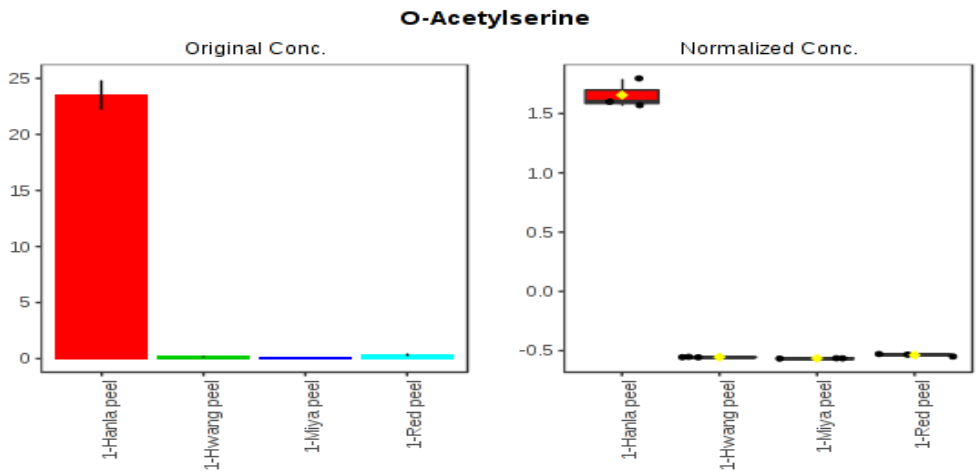
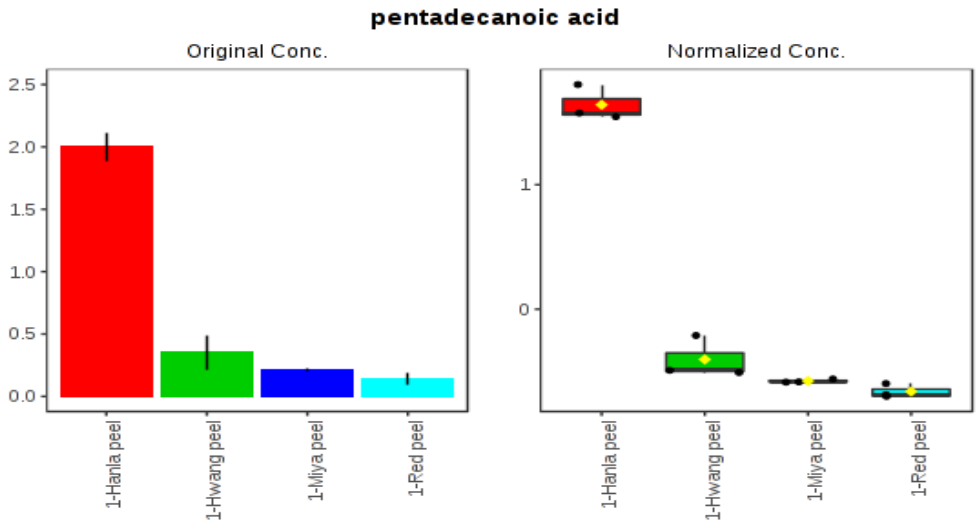


Figure 16. compound to specify Hanlabong peel


O-Acetylserine
Original Conc.
Normalized Conc.
25
20
15
10
5
0
1.5
1.0
0.5
0.0
-0.5
1-Hanla peel
1-Hwang peel
1-Mlya peel
1-Red peel


pentadecanoic acid
Original Conc.
Normalized Conc.
2.5
2.0
1.5
1.0
0.5
0.0
1
0
1-Hanla peel
1-Hwang peel
1-Mlya peel
1-Red peel

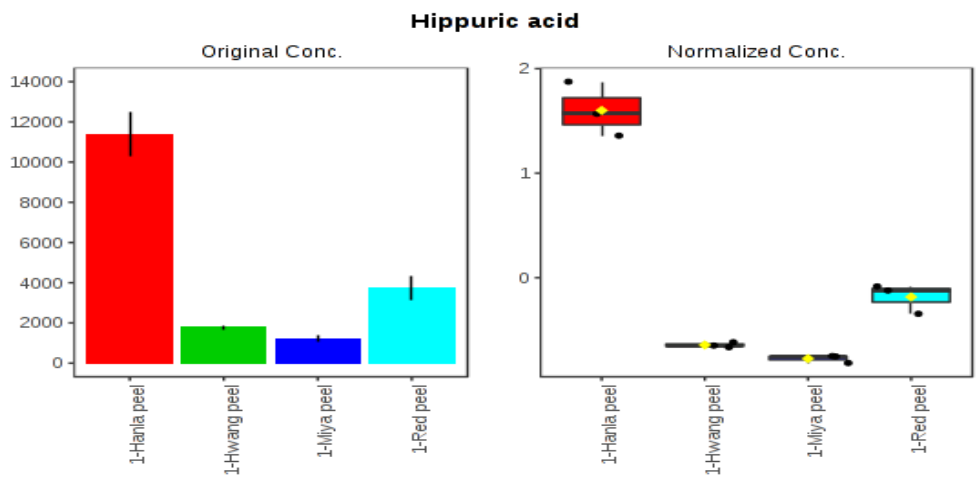
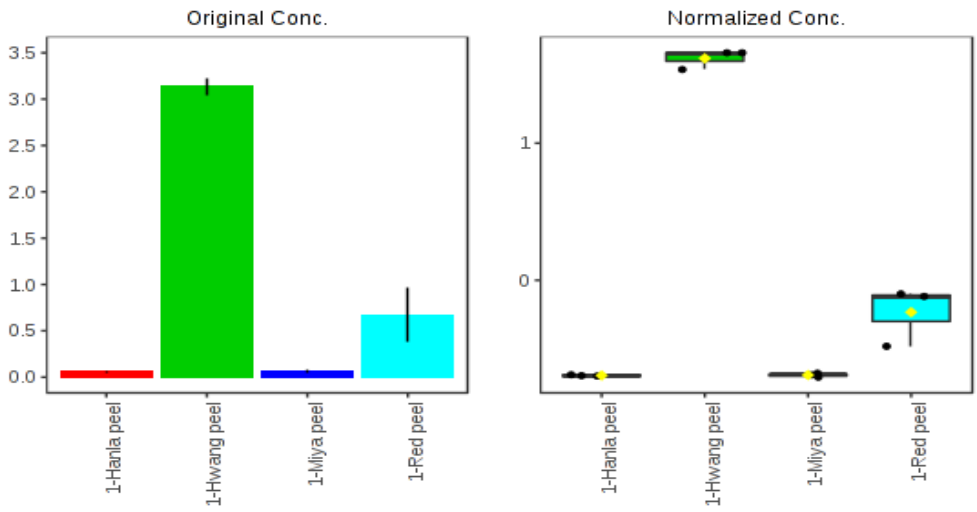
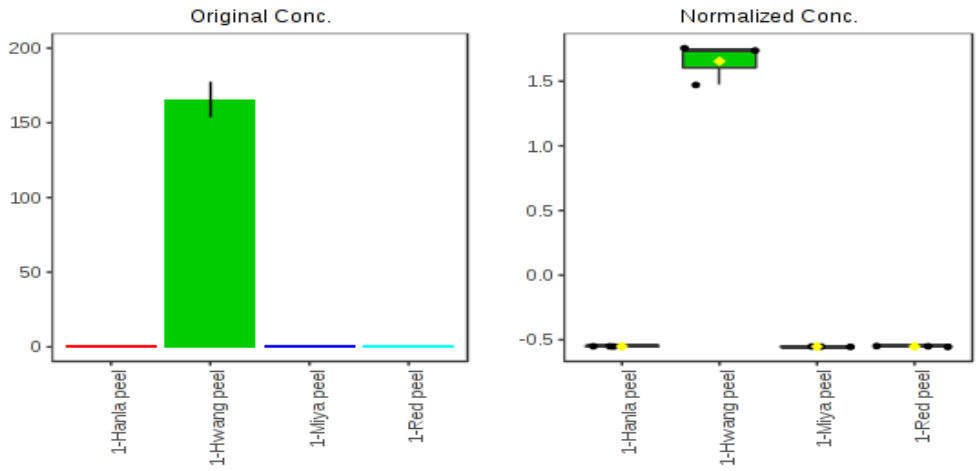

Hippuric acid
Original Conc.
Normalized Conc.
14000
12000
10000
8000
6000
4000
2000
0
2
1
0
1-Hanla peel
1-Hwang peel
1-Mlya peel
1-Red peel

Figure 17. compound to specify Hwangkeumhyang peel

oxamic acid



Putrescine



Xanthosine

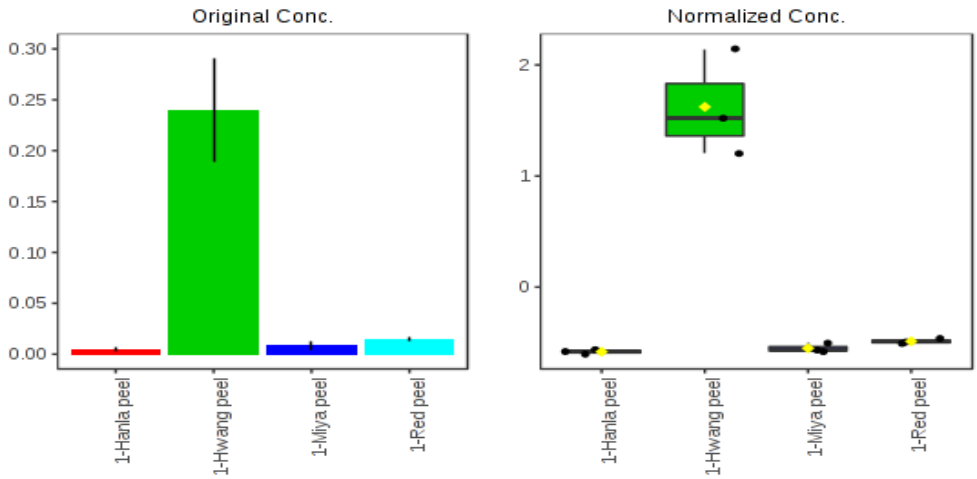
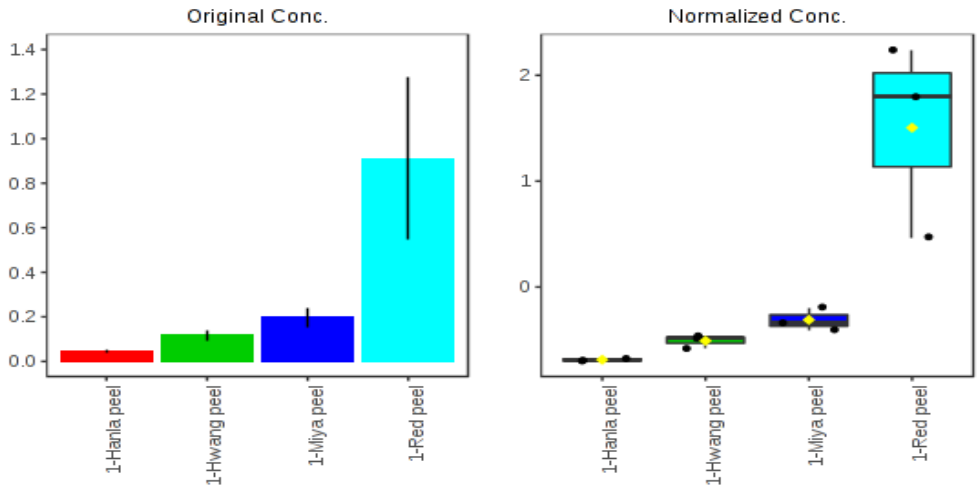
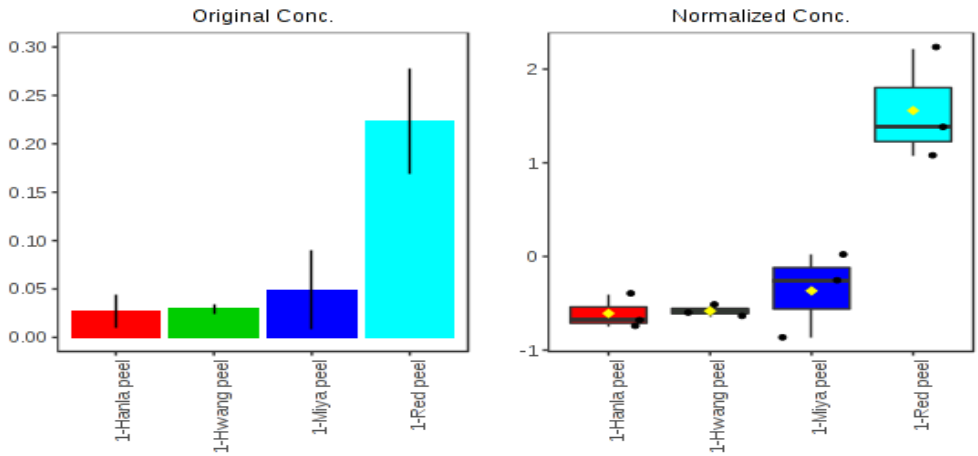


Figure 18. compound to specify Redhyang peel

Cystathionine



Spermidine



Thymidine

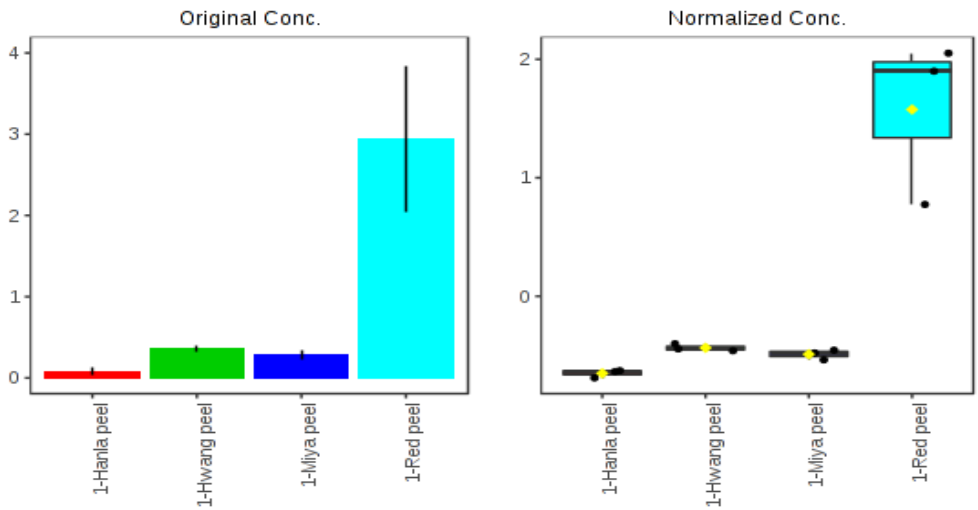
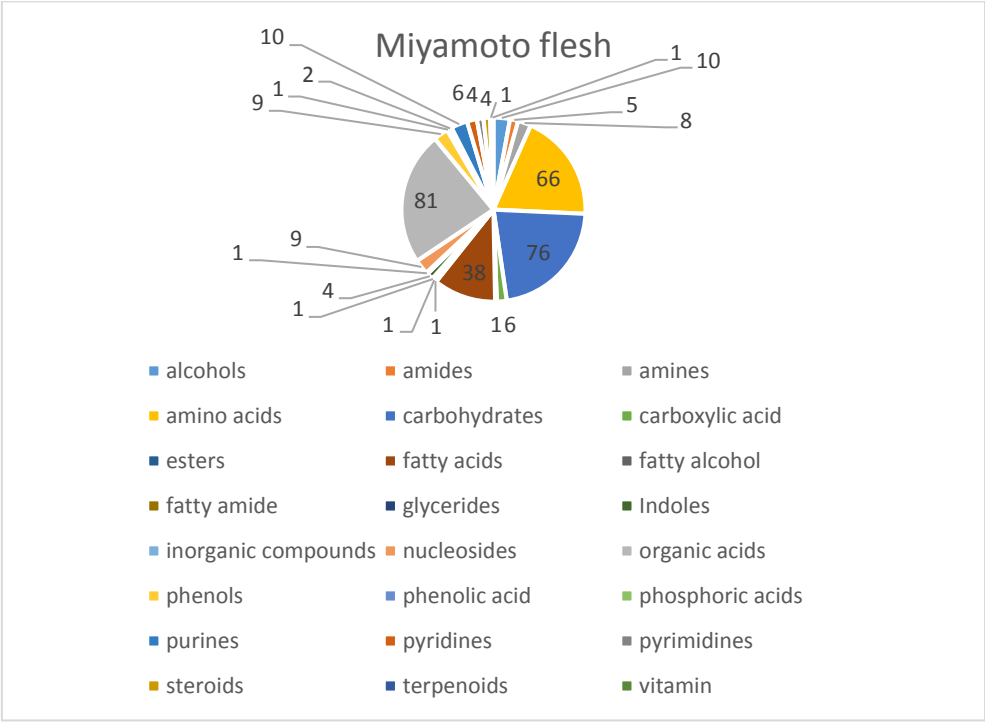


Figure 19. Classification of total compounds in (A) Miyamoto flesh,

(B) Miyamoto peel

(A)


Miyamoto flesh
10
2
9
1
6
4
4
1
1
10
5
8
66
81
76
38
9
1
4
1
1
16
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

(B)

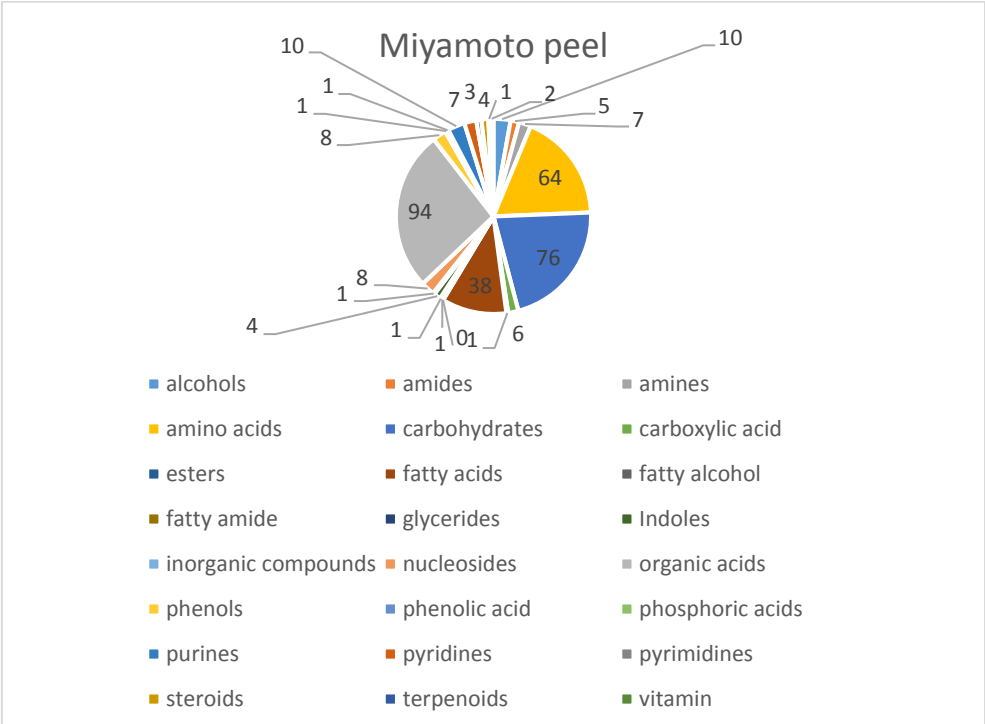
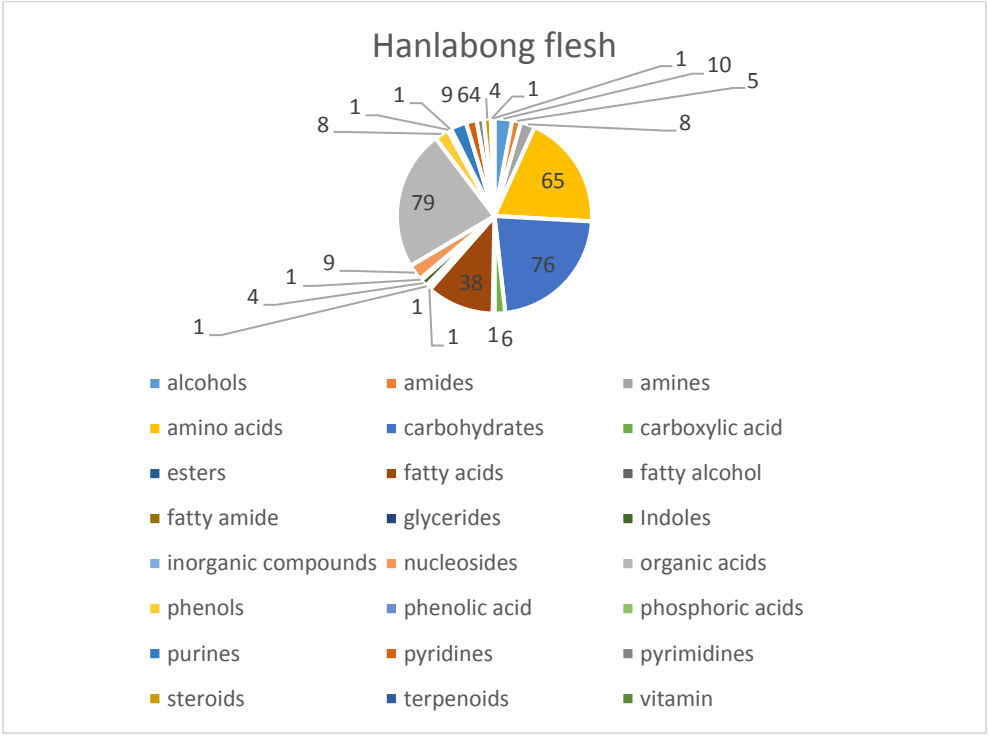

Miyamoto peel
10
1
1
8
7
3
4
1
2
5
7
10
64
94
76
38
1
8
4
1
1
0
1
6
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

Figure 20. Classification of total compounds in (A) Hanlabong flesh, (B) Hanlabong peel

(A)


Hanlabong flesh
1
10
5
1
9
6
4
4
1
1
8
8
65
79
76
9
1
4
38
1
1
1
1
6
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

(B)

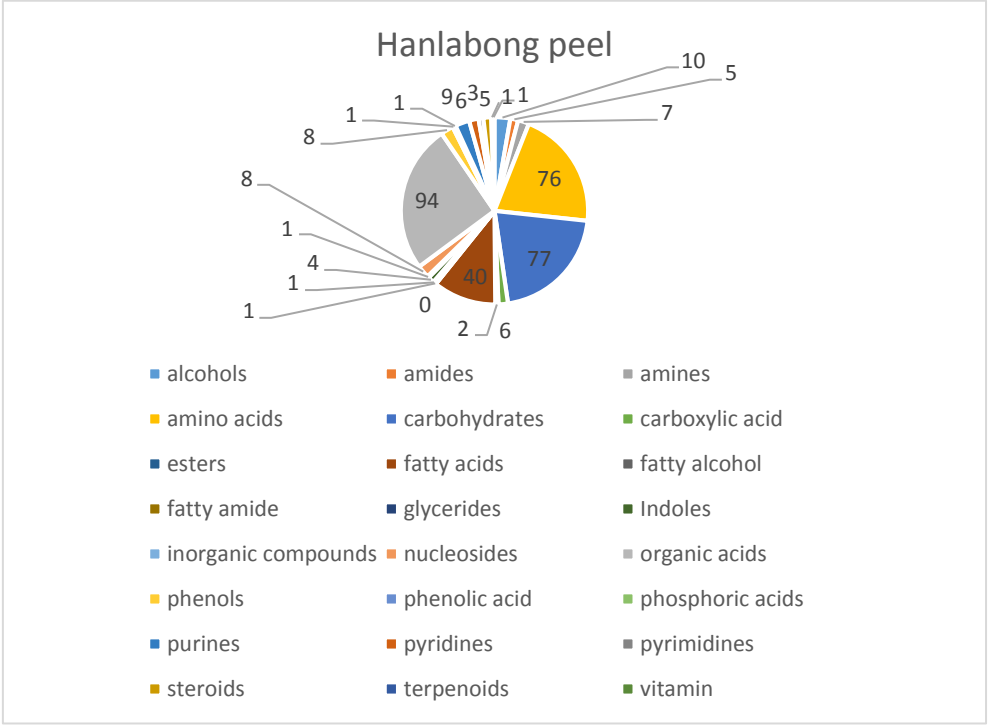
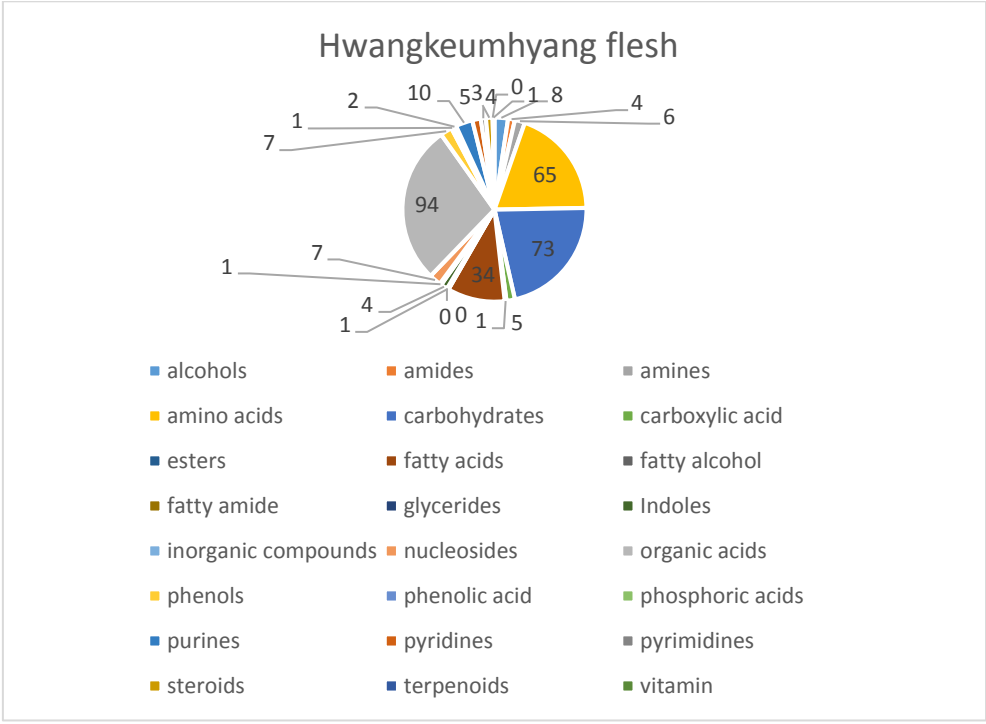

Hanlabong peel
10
5
1
9
6
3
5
1
1
1
7
8
8
76
94
1
4
77
1
40
1
0
2
6
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

Figure 21. Classification of total compounds in (A) Hwangkeumhyang flesh, (B) Hwangkeumhyang peel

(A)


Hwangkeumhyang flesh
2
10
5
3
4
0
1
8
4
6
1
7
65
94
73
7
1
34
4
1
0
0
1
5
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

(B)

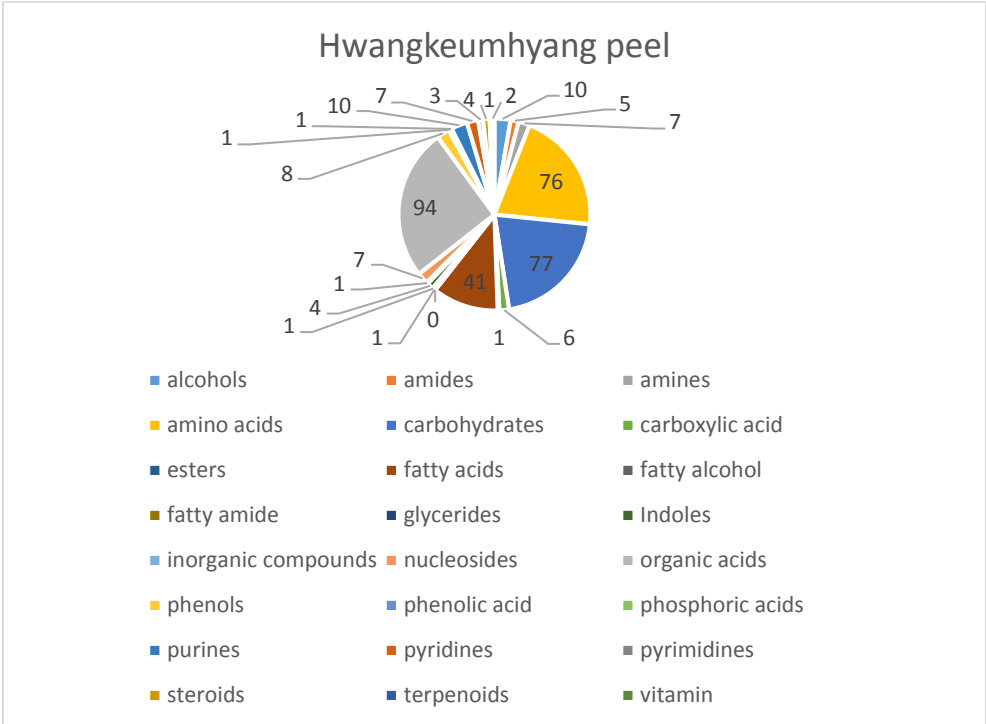
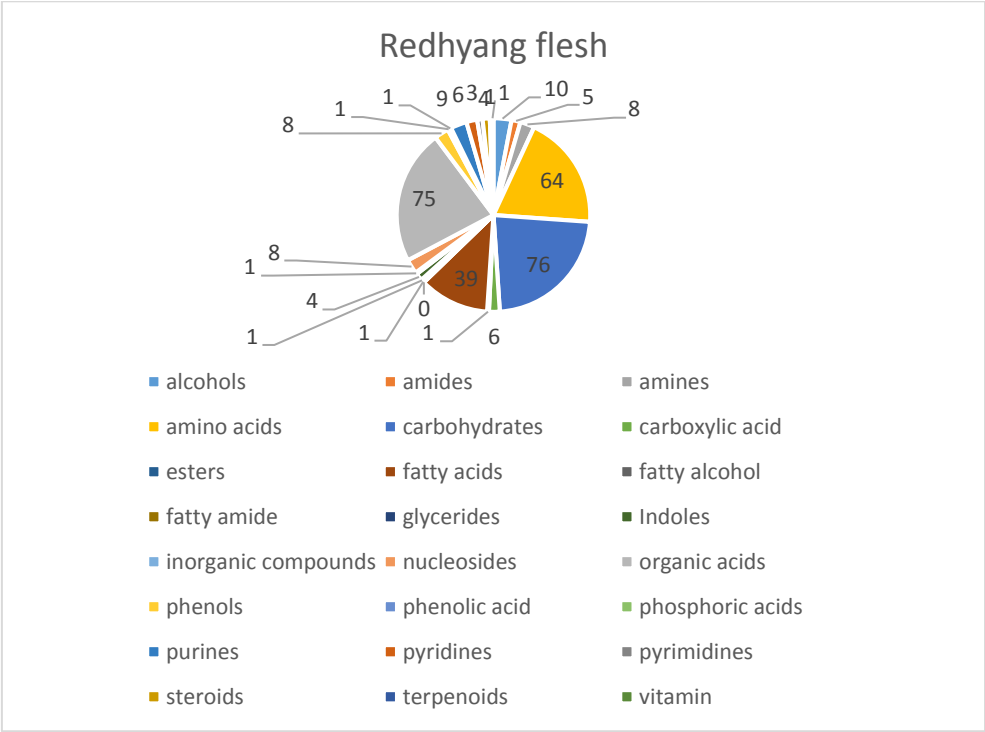
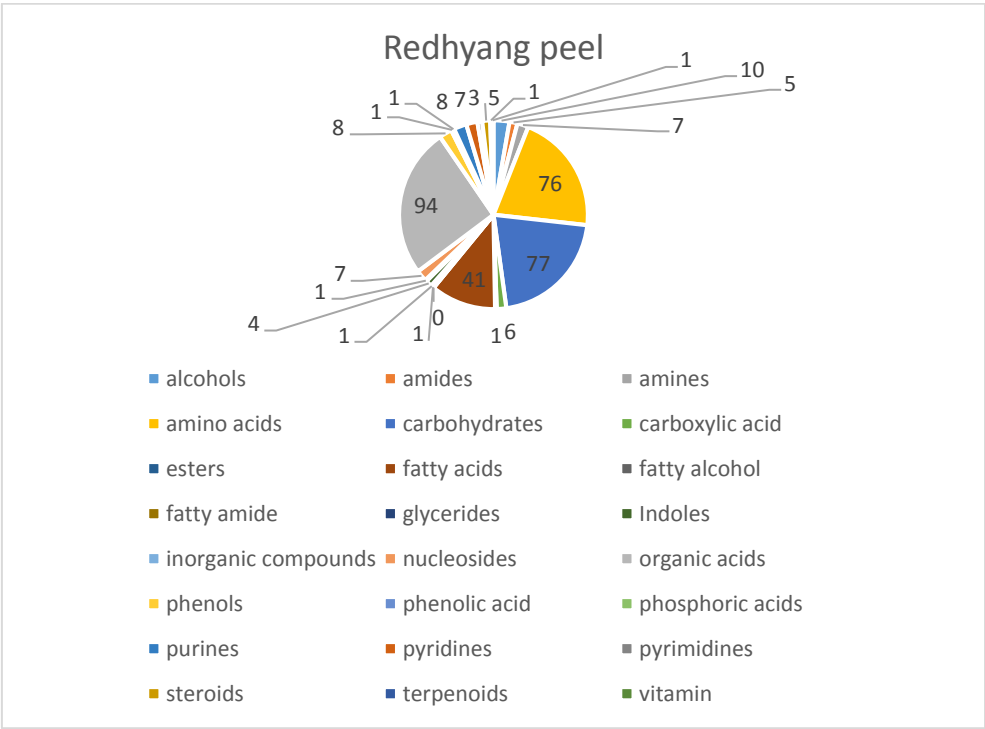

Hwangkeumhyang peel
10
7
3
4
1
2
10
5
7
1
1
8
76
94
77
7
1
4
1
41
0
1
1
6
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

Figure 22. Classification of total compounds in (A) Redhyang flesh, (B) Redhyang peel

(A)


Redhyang flesh
1 1 9 6 3 4 1 10 5 8
8 1
64
75
76
39
1 8
4
0
1 1 1 6
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

(B)


Redhyang peel
1 10 5
1 1 8 7 3 5 1
8 7
76
94
77
41
7
1
4
1 1 0 16
alcohols
amides
amines
amino acids
carbohydrates
carboxylic acid
esters
fatty acids
fatty alcohol
fatty amide
glycerides
Indoles
inorganic compounds
nucleosides
organic acids
phenols
phenolic acid
phosphoric acids
purines
pyridines
pyrimidines
steroids
terpenoids
vitamin

Discussion

Figure 1 shows the results of scanning a new standard to add to an existing method. Based on the MRM conditions in Table 2, 16 standard methods containing 378 compounds were added to the metabolite method. Figures 6 and 12 show the results of ANOVA (ANalysis Of VAriance), and AVONA is an analysis method to verify if the mean of the dependent variable differs between groups. Reliable when P-value is less than 0.05. Figures 6 and 13 shows that results of running a statistical program, PCA, based on the data of GC-MS/MS analysis. In the PCA score plot picture shows that each samples are located at each location. In the case of PLS-DA, data can be taken more selectively than PCA and can be distinguished more easily. VIP methods is one of the ways to select useful data. So that low-quality data can be discarded and compared to a specific sample based on high-quality data. As a result, based on the selected data, samples can be specified more accurately and easily. The heatmap in figure 7 shows the classification of the lines based on the color differences. The closer to dark red, the validity of the data value. The closer to dark blue, the less data value. Based on the results in Figure 8 to 11, the data were obtained. Each sample which has a lot of ingredients can make them to distinguish different samples. In the flesh of Miyamoto, n-acetylneuraminic acid, glucono-1,5-lactone and inositol were present more than other samples and could be specified. However, Miyamoto was mostly sugar-rich than special ingredients. gluconic acid, 2-deoxy-glucose and 4-hydroxyphenylpyruvic acid were presented in flesh of Hanlabong. There were more tyramine, octopamine and coniferyl alcohol in the flesh of Hwangkeumhyang than other samples. There were more adenine, 2-aminooctanoic acid and valproic acid in Redhyang flesh than other samples.

The heatmap in figure 14 also shows the classification of the lines based on the color differences, as in figure 7. According to the results in Figures 15 to 18, in the peel of Miyamoto, methionine and tyramine, were presented more than other samples. Like as flesh, there were not many special ingredients compared to other samples. There were more o-acetylserine, pentadecanoic acid, hippuric acid in Hanlabong peel than other samples. In the peel of Hwangkeumhyang, oxamic acid, putrescine, xanthosine were presented more than other samples, In case of Redhyang peel, cystathionine, spermidine and thymidine were presented more than other samples. As a result, hwangkeumhyang was found to have the highest content in both flesh and peel.

Conclusion

Based on the process of the PLS-DA and VIP, eight different compounds and characteristics were analyzed. Only PCA process would have been difficult to effectively analyze over 390 compounds. Derivation analysis via GC-MS/MS has the advantage of being able to compare and analyze more than 390 substances in one sample. In addition, a lot of information can be obtained with a short time and a minimum of effort, and ANOVA, PCA and PLS-DA statistics programs together, we can reduce the time required to analyze large amounts of data. Through the experimental methods used in this experiment, we could classify similar or less similar strains based on the compounds of the fruits to be compared and analyzed. Based on these results, it can be effective in improving fruit varieties or breeding high value fruits.In addition to foods such as fruits, it may be possible to analyze substances in various fields. In particular, it would be effective to conduct a comparative analysis of compounds using metabolomix, and to analyze unknown samples.

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Abstract in Korean

서울대학교 대학원

농생명공학부 응용생명화학전공

이강헌

본 연구는 한국에서 많이 소비되는 과일 중 하나인 감귤류 중 미야모토귤, 황금향, 한라봉, 레드향의 과육과 껍질을 분리한 후, 대사체학 (metabolomics) 을 적용하여 각각 생체 대사물질을 비교 하였으며, 분석 감도를 향상 시켜주고, 더 나은 분리를 할 수 있게 해주는 유도체화 방법을 이용하여 GC-MS/MS 에서 분석하였다. 분석한 자료를 바탕으로 통계 도구인 Principal Component Analysis (PCA) 의 score scatter plot 과 ANOVA를 사용하여 1차 적으로 선별해 준 후, Partial least squares Discriminant Analysis (PLS-DA) 를 이용하여 보다 세밀하게 감귤류 각각의 시료를 특정하는 물질을 확인하였다. 미야모토귤의 경우 과육에서 n-acetylneuraminic acid, glucono-1,5-lactone 그리고 inositol, 과피의 경우 methionine과 tyramine들이 이 시료를 특정하는 물질로 나타났고. 한라봉의 경우 과육에서 gluconic acid, 2-deoxy-glucose 그리고 4-hydroxy-phenylpyruvic acid이 과피에서 o-acetylserine, pentadecanoic acid, hippuric acid 으로 나타났으며, 황금향의 경우 과육에서 tyramine, octopamine 그리고 coniferyl alcohol이, 과피의 경우 xamic acid, putrescine, xanthosine등이 다른 시료에 비해 많이 나타났다. 레드향의 경우 과육에서 adenine, 2-aminooctanoic acid and valproic acid, 과피에서 cystathionine, spermidine 그리고 thymidine이 시료를 특정하는 물질로 나타났다.

주요어: ANOVA, derivatization, GC-MS/MS, Hwangkeumhyang, Hanlabong Metabolomics, Miyamoto, PCA, PLS-DA, Redhyang

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첫만남부터 헤어질때까지 항상 예뻐해 주시고 좋은 말 만 해주신 희원이형님 오늘따라 더 보고 싶네요. 그리고 내 동기, 예림이랑 수연이, 항상 좋은 일만 있자. 특히 예림아 너한테는 말로 다 못할 정도로 고마운 것도 미안한 것도 너무 많아서 무슨 말을 해야 될지 모르겠네. 결혼 축하한다.

그리고 정학아 가끔씩 내가 빛정학이라고 그랬는데 진심이야. 정말 너무 고마워. 네 배려 덕분에 실험실 적응도 잘할 수 있었고, 네 덕에 졸업도 하게 됐네. 고마운 마음 잊지 않을게

우리 실험실 넘버 2 라고 할 때마다 손사래 치는 은영이, 네가 서글서글하게 말도 잘 걸어주고 항상 밝아서 좋은 기운 많이 받은거 같다. 힘들겠지만 지금처렁 많이 웃고 밝은 기운 퍼뜨리면 곧 좋은 소식 있을 거야

원수는 타지에서 공부하느라 외롭고 힘들텐데 실험도 공부도 열심히 잘하는 거 보면 대견하다. 끼니 거르지 말고 건강 잘 챙기고, 그렇게 바라는 좋은 소식 빨리 오길 기도해줄게

락도야 일단 취업 정말 축하한다. 이제 학생신분에서 사회인으로 넘어가는 시점인데, 넌 싹싹하고 총명하니 회사 생활도 전혀 문제 없을 거 같다. 승승장구하고 그 동안 너랑 이야기 나누면서 힐링도 많이 됐다 고맙고 나중에 밖에서 소주한잔 하자.

예의 바르고 착한 보은이, 한번씩 힘들어 할 때마다 안쓰러웠는데, 마음에 와닿게 도움 준 적이 없는 것 같네, 넌 잘 하고 있고 더 잘 할 수 있어 파이팅, 힘내고 지지마라

마이는 대화를 별로 안해봐서 잘 모르겠네 너 보면 아랍어로 유투브 보면서 혼자 웃는거 만 생각난다. 졸업 잘해

마지막 희주, 극과 극은 통한다더니 막내랑 노인이 통할줄이야. 네 덕에 많이 웃고 재밌었어. 처음엔 너무 어려서 어떻게 대해야 할지 몰랐는데 네가 서슴없이 다가와서 빨리 친해질 수 있었던거 같애. 너의 당당함과 뻔뻔함은 정말 최고야. 행복한 일만 있어라 막내 꼬꼬마