약학석사학위논문

Studies on rice metabolomics in different degrees of milling

도정도에 따른 쌀 대사체의 변화 연구

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DONG ZIYUAN의 석사학위논문을 인준함

2017 년 8 월

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Abstract

Studies on rice metabolomics in different degrees of milling

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Nutrients in rice have been a hot topic in scientific studies for a long time. As an issue of debate in these studies, a quite amount of studies indicate that brown rice has greater benefit of nutritional value than white rice. Nevertheless, nutritional components of rice with remarkable variations between brown rice and white rice which could verify the proposition proposed above, have not been investigated comprehensively. In addition, the variation tendency requires to be described not only according to the two types of rice (brown rice and white rice) but also products of rice in different degrees of milling (DOM). This assay examined variations of rice components among different DOM using untargeted metabolomics approach. Rice processed in DOM values of 0, 5, 7, 9 and 11 were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) and gas chromatography-mass spectrometry (GC-MS). To detect nutritional components of rice which exhibit significant changes among different DOM, principal component analysis (PCA) and

partial least squares-discriminant analysis (PLS-DA) were applied. On account of the analysis of the results, we found that the contents of sugars and sugar alcohols decreased with the rise of DOM due to the lack of bran layer. While the contents of phospholipids had rising tendency with the increase of DOM. In conclusion, in contrast to the common opinion, our results revealed that the nutritional values of rice changed in various situations. Rice in different DOM provided the maximum benefits in different cases. Accordingly, the variation tendency and regularity of rice components among different DOM had great contribution to the rational adjustment of rice production and consumption.

Keywords: Rice metabolomics; Degree of milling; Gas chromatography-mass spectrometry (GC-MS); High-performance liquid chromatography-mass spectrometry (HPLC-MS); Sugar; Phospholipid.

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1. Introduction

Rice has been used as staple food for over half of the world's population all the time, especially in Asia, since it is competent to provide enough calories for people throughout the whole year[1]. Accordingly, a large number of studies have kept on the componential analysis and quality improvement of rice. Based on the studies investigated before, all varieties of rice are high in carbohydrates, fibers, vitamins and minerals as well as proteins[1, 2]. Commonly, there are two types of rice product in rice consumption, brown rice and white rice. Brown rice is unpolished whole grain rice that is processed by removing only the hull. After that, the bran layer and germ of brown rice are peeled off through milling to produce white rice. Thus, white rice is more commonly used in daily life owing to its excellent appearance, texture and taste[3]. By contrast, it has been examined that brown rice contains more minerals, vitamins, and proteins, which are abundant in the bran layer and germ[1, 4]. Coupled with its low supply and difficulty of storage and transport, brown rice is by far more expensive than white rice[1]. As stated above, these studies have brought powerful influences on rice production, consumption and trade. However, variations of nutritional components between brown rice and white rice have not been investigated in all directions. For this reason, more detailed and systematical studies on the nutritional value evaluation of rice in different forms become in urgent need.

The nutritional values of rice in different forms mainly depend on the variation tendency and regularity of nutritional components. To describe the variation tendency and regularity comprehensively, rice cannot be simply categorized into two types, brown rice and white rice. Specifically, as an influential factor of nutritional components in rice, different degrees of milling (DOM) create numerous intermediate products of rice. Moreover, these intermediate products show different textures,

contents of nutritional components and variation characteristics during the milling process. According to this character of rice, recently, DOM of rice becomes an issue open to debate. Thus far, studies on the DOM of rice have concentrated only on taste differences and loss of proteins, vitamins, and minerals in the germ and bran layer of rice during the milling process[4, 5]. Nevertheless, variations of nutritional components in endosperm, particularly phospholipids, which have abundant benefits for health[6, 7], gained few attentions by the public. Therefore, nutritional value evaluations of rice in different DOM deserve more comprehensive investigations.

Rice metabolomics is a comprehensive analysis technology of metabolites in rice, which investigates the amounts and variations of rice metabolites through quantitative and qualitative analysis[8]. In this study, three different cultivars of rice in different DOM were analyzed by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-mass spectrometry (HPLC-MS) based on untargeted metabolomics approach. In detail, three different cultivars of rice in DOM values of 0 (brown rice), 5, 7, 9 and 11 were obtained from Korean local markets. After aligning metabolomic data acquired before, multivariate statistical analysis methods were employed to understand the comprehensive variations in the detected metabolites among various rice samples. More specifically, principal component analysis (PCA) was applied to depict the dissimilarity among rice in different DOM. Meanwhile, the nutritional components of rice with remarkable variations among different DOM were picked out by partial least squares discriminant analysis (PLS-DA) combined with one-way analysis of variance (ANOVA). Eventually, the variation tendency and regularity of the nutritional components which have remarkable variations among different DOM were described by compare of their concentrations. On the basis of this study, the nutritional value evaluations of rice in different DOM were optimized. Furthermore, rice production and consumption can be adjusted to optimum DOM according to certain specific demands.

2. Experiment

2. 1. Sample collection and pretreatment

Three different cultivars of Korean rice, as representative short/medium grain rice cultivars which named Choochung, Shindongjin, and Ode, in DOM values of 0, 5, 7, 9, and 11, were purchased from local markets in Korea. All rice samples were ground to fine powder and freeze-dried in the dark for two days. Subsequently, all the samples were stored at -70°C before study to avoid metabolic changes.

2. 2. Chemicals and materials

2. 2. 1. Chemicals

Chemicals used in GC-MS analysis

- Chloroform (J.T. Baker, Phillipsburg, NJ, USA)
- Methanol (J.T. Baker, Phillipsburg, NJ, USA)
- Water (J.T. Baker, Phillipsburg, NJ, USA)
- Methoxyamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA)
- Pyridine (Sigma-Aldrich, St. Louis, MO, USA)
- *N*,*O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (Sigma-Aldrich, St. Louis, MO, USA)

All the reagents are in analytical grade.

Chemicals used in HPLC-MS analysis

- Isopropanol (J.T. Baker, Phillipsburg, NJ, USA)
- Acetonitrile (J.T. Baker, Phillipsburg, NJ, USA)
- Water (J.T. Baker, Phillipsburg, NJ, USA)
- Formic acid (Sigma-Aldrich, St. Louis, MO, USA)

All the reagents are in analytical grade, and all the solvents are in HPLC grade.

Standards used for calibration

- Caffeine (Sigma-Aldrich, St. Louis, MO, USA)

2. 2. 2. Experimental supplies

- Adjust Pipette (0.5 ~ 10 μL, 20 ~ 200 μL, 100 ~ 1000 μL, Eppendorf AG, Hamburg, Germany)
- Pipette Tips (0.5 ~ 10 μL, 20 ~ 200 μL, 100 ~ 1000 μL, Eppendorf AG, Hamburg, Germany)
- Safe-lock Tube (2 mL, Eppendorf AG, Hamburg, Germany)
- Conical Tube (50 mL, SPL Life Sciences Co. Ltd)
- Clear Crimp Top Fixed Insert Vial (2 mL, Agilent, Santa Clara, CA, USA)
- Clear Wide Opening Screw Top Vial (2 mL, Agilent, Santa Clara, CA, USA)
- Sterile Hypodermic Syringe (1 mL, Korea Vaccine Co. Ltd)
- PTFE Syringe Filter (0.20 μm, Advantec, Japan)
- Vortex Mixer (Vortex Genie 2)
- Centrifuge (Eppendorf AG, Hamburg, Germany)
- SpeedVac Vacuum Concentrator AES2010 (Savant, Holbrook, NY, USA)

- Vacuum Ovens OV-01 (Lab Companion)
- Chemical-free Freeze Dryer (-120°C, Operon)

2. 2. 3. Analytical instruments

- GC-MS OP2010 (Shimadzu, Kyoto, Japan)

  DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness)
- HPLC (Agilent) –MS (Q-TOF 6530 MS, Agilent, USA)

  Acquity™ UPLC column (1.7 μm; 2.1 mm × 100 mm, BEH C18)

2. 3. Sample preparation

2. 3. 1. GC-MS experimental method

The method of sample extraction and preparation was in accordance with previously developed study[9], as shown in Figure 1. To be specific, 100 mg of rice powder mixed with 0.5 mg of caffeine which was employed as the internal standard, were extracted with 1 mL solvent mixture consisted of chloroform: methanol: water (1:2.5:1, volume ratio). The extraction was performed using sonication treatment at room temperature for 30 min. Then, the extract was centrifuged at 16,000 g (g = 9.8 m/s^2) for 5 min and 500 µm supernatant of the methanol/water phase was transferred to a 2 mL clear crimp top fixed insert vial. Therewith, the supernatant extract was dried using a SpeedVac vacuum concentrator AES2010 at 5,000 g and 45°C for 10 h. After that, the dried sample was oximated with 80 µL of methoxyamine hydrochloride dissolved in pure pyridine (15 mg/mL) and incubated at 30°C for 90 min. Therewith, 100 µL of BSTFA containing 1% TMCS was mixed into sample and then the mixture was kept at 60°C for 15 min. The vial was covered with a cap and waiting for injection.

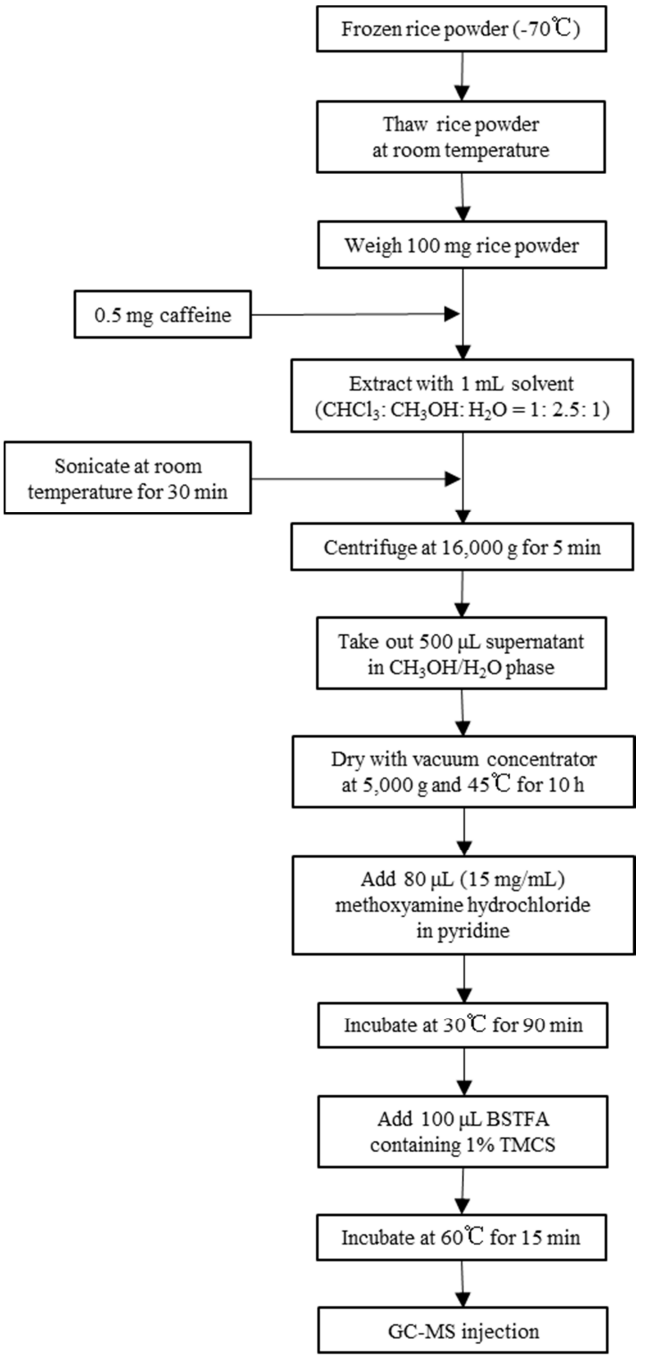


Figure 1. Flowchart of sample preparation for GC-MS analysis.

2. 3. 2. HPLC-MS experimental method

The optimized extraction method for plant metabolomics, which has been investigated by previous study, was modified suitably to extract metabolites of rice samples in this study[10], as shown in Figure 2. In brief, 100 mg of rice powder was mixed with 1 mg of caffeine as the internal standard to evaluate the reproducibility and stability of HPLC-MS analysis. The mixture was then extracted with 1 mL of 75% isopropanol and sonicated at 90°C for 2 h, followed immediately by the centrifugation at 12,000 g for 5 min. The supernatant was removed from the crude extract and filtered with a 0.2 μm PTFE filter. The fine extract was collected for injection. Especially, a randomized sequence was applied in HPLC-MS analysis.

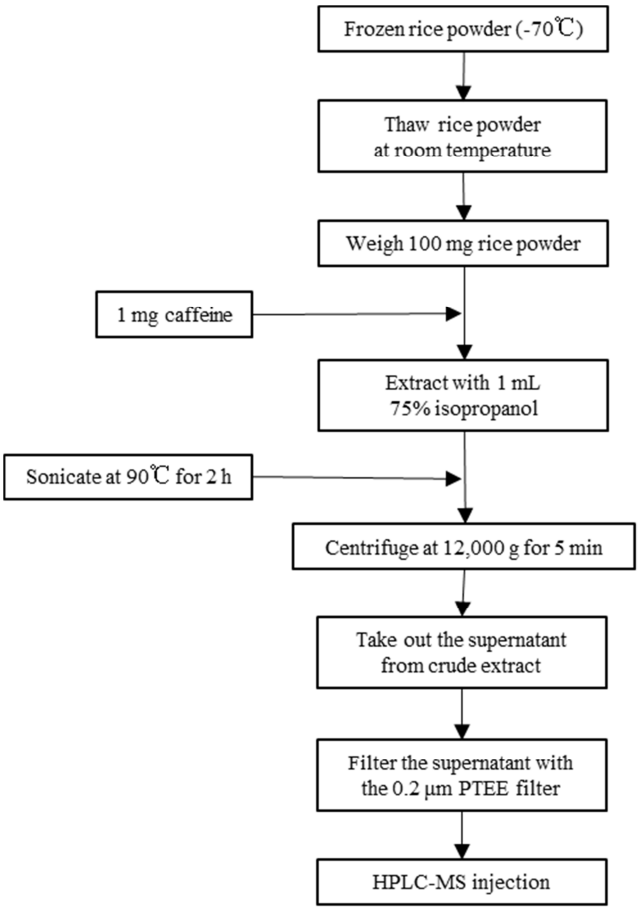


Figure 2. Flowchart of sample preparation for HPLC-MS analysis.

2. 4. Instrumental analysis

2. 4. 1. GC-MS analysis

GC-MS analysis was performed by the GCMS-QP2010 system. The chromatographic separation was accomplished with a DB-5 capillary column. The analysis conditions are listed in Table 1. The GC oven temperature was firstly held at 60°C for 5 min, then the temperature rose to 300°C at a constant velocity of 6^{o}C/min and held at the final temperate for 10 min. 1 μL of sample was injected using 1:2 split-mode at 300°C and helium was used as the carrier gas under a constant flow of 1.0 mL/min. The ion source temperature and the interface temperature were 200°C and 300^{o}C, respectively. The ionization energy was 70 eV in electron impact mode. The mass spectrometer was operated in scan mode from *m/z* 40 to 500. The sequence of the sample in GC-MS analysis was set at random.

Instrument	GC-MS OP2010 (Shimadzu, Kyoto, Japan)
Column	DB-5 capillary column (30 m × 0.25 mm, 0.25 μm)
GC parameters	Injection temperature: 300℃ Injection mode: Split (1:2) Injection volume: 1 μL Carrier gas: He Carrier gas flow: 1.0 mL/min Oven program: Temperature / Hold Time / Rate 60℃ / 5 min / 6℃/min 300℃ / 10min / End
MS parameters	Ion source temperature: 200℃ Interface temperature: 300℃ Ionization mode: 70 eV Mass range: 40 – 500 *m/z*

Table 1. GC-MS analysis conditions.

2. 4. 2. HPLC-MS analysis

HPLC-MS analysis was performed by using an Agilent HPLC system equipped with an Acquity™ UPLC column (1.7 μm; 2.1 mm × 100 mm, BEH C18) and coupled to an Agilent Q-ToF 6530 MS. The analysis conditions are listed in Table 2. The column temperature was maintained at 40°C cooperated with the flow rate of 0.17 mL/min. As a fixed volume of injection, 5 μL of each sample was injected and separated by the following gradient method with linear changes. Solvent A (water + 0.1% formic acid) and Solvent B (acetonitrile + 0.1% formic acid): 0 min, 100 % A, 0% B; 5 min, 70 % A, 30% B; 15 min, 30 % A, 70% B; 25 min, 20 % A, 80% B; and 27 min, 0 % A, 100% B. As the equilibration time, 10 min of column equilibration was executed after each sample injection. Furthermore, the mass spectrometer was operated in ESI negative ionization mode, with the scan mass range of *m/z* 50 ~ 1500. Flow injection of the lock mass standard was applied in each spectrum to ensure the accuracy of the *m/z* value.

Instrument	HPLC (Agilent) MS (Q-TOF 6530 MS, Agilent, USA)
Column	Acquity™ UPLC column (1.7 μm; 2.1 mm × 100 mm, BEH C18)
LC parameters	Injection volume: 5 μL Column oven temperature: 40°C Flow rate: 0.35 mL/min Gradient condition: Solvent A (water + 0.1% formic acid) Solvent B (acetonitrile + 0.1% formic acid) 0 min 100 % A; 5 min 70 % A; 15 min 30 % A; 25 min 20 % A; and 27 min 0 % A
MS parameters	Ionization mode: negative mode Mass range: 50 - 1500 *m/z* Dry gas: 8.0 L/min Dry temperature: 200°C Nebulizer pressure : 1.2 bar

Table 2. HPLC-MS analysis conditions.

2. 5. Data processing

2. 5. 1. GC-MS data processing

The original data of GC-MS analysis were exported in *.CDF format for subsequent data processing. As shown in Figure 3, the data alignment was accomplished using MZmine 2.19 and the detailed processes and algorithms were listed as follows: the centroid algorithm was employed in Mass detection; the baseline cut-off algorithm was applied to deconvolution; the RANSAC aligner was used for data bucketing and finally the gap filler with the same RT and *m/z* range was used to fill missing values[11]. The data alignment parameters of MZmine 2.19 are listed in Table 3. Additionally, the Automated Mass Spectral Deconvolution and Identification System (AMDIS) was performed to group the fragment ions as well as precursor ions with mass spectra. Prior to the statistical analysis, the aligned data were processed using log-transformation and Pareto scaling. The multivariate statistical analysis then came into effect on the processed data. The univariate and multivariate analyses were performed regarding the typical workflow and guideline of MetaboAnalyst 3.0[12]. Specifically, PCA and PLS-DA were used as the classification methods for discrimination. The variable importance in projection (VIP) score and false discovery rate (FDR) were then applied to select markers. Subsequently, the marker candidates were found out with NIST08 database. The markers were finally confirmed by the comparison of mass spectra and chromatographic retention time between standards and rice samples. The workflow of data processing is shown in Figure 4.

2. 5. 2. HPLC-MS data processing

The raw data of HPLC-MS analysis were collected in mzData format and then processed by MZmine version 2.19 (Figure 3)[11]. In detail, the processes and algorithms used for HPLC-MS were roughly the same as those in GC-MS analysis, expect the minor modification on certain parameters according to the differences between the GC-MS and HPLC-MS platforms. The data alignment parameters of MZmine 2.19 are listed in Table 3. Moreover, the mass spectra of fragment ions as well as the corresponding precursor ions were collected. Following data aligning, the data were processed using log-transformation and Pareto scaling before statistical analysis. The univariate and multivariate analyses were then performed by MetaboAnalyst 3.0 with the typical workflow and guideline[12]. PCA was employed in classifying various samples, besides PLS-DA was mainly applied to select the markers with FDR and VIP score. The markers were identified by their fragmentation pattern of precursor ions using our internal library and the METLIN metabolite database (http://metlin.scripps.edu/)[13]. The workflow of data processing is shown in Figure 4.

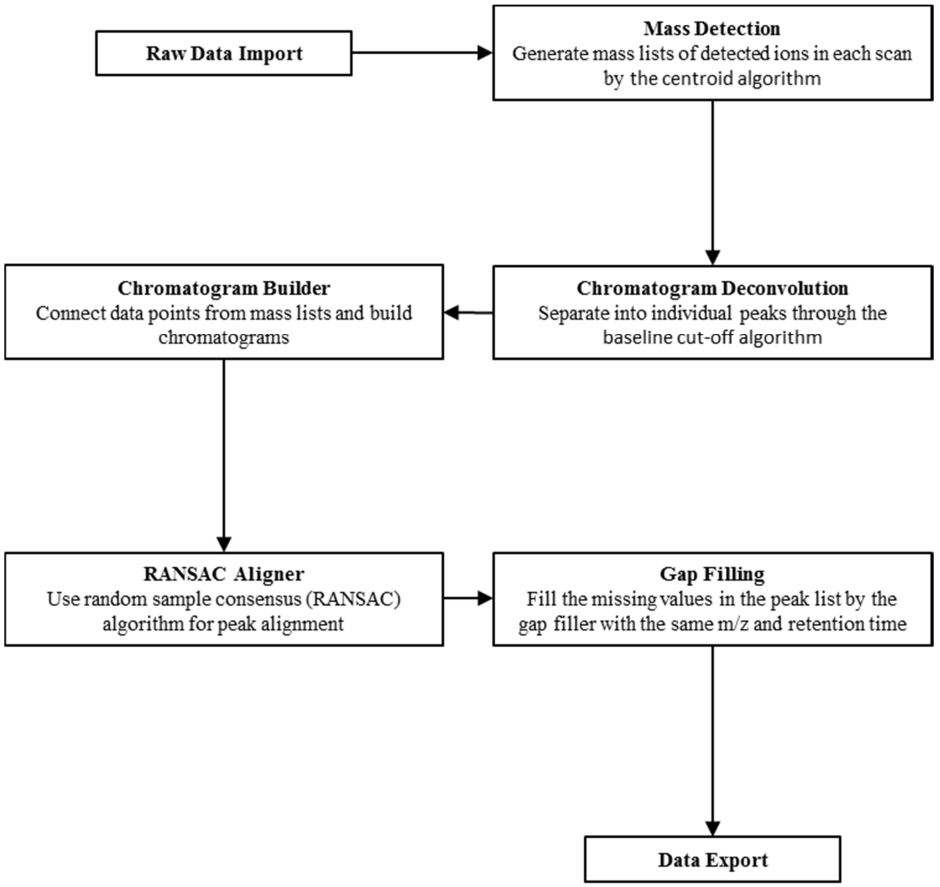


Figure 3. The data alignment process of MZmine 2.19.

	GC-MS	HPLC-MS
Conversion	Noise level: 5×10^3	Noise level: 5×10^4
Chromatogram construction	Minimum height: 5×10^3 m/z tolerance: 1×10^{-4}	Minimum height: 5×10^4 m/z tolerance: 3×10^{-5}
Peak recognition	Minimum peak height: 1×10^4 Derivative threshold level: 20%	Minimum peak height: 5×10^4 Derivative threshold level: 20%
Peak alignment	m/z tolerance at 1×10^{-4} Retention time tolerance: 0.05 min RANSAC iterations: 1×10^5	m/z tolerance at 3×10^{-5} Retention time tolerance: 0.1 min RANSAC iterations: 1×10^5
Peak gap filling	m/z tolerance: 2×10^{-4}	m/z tolerance: 1×10^{-4}

Table 3. The data alignment parameters of MZmine 2.19.

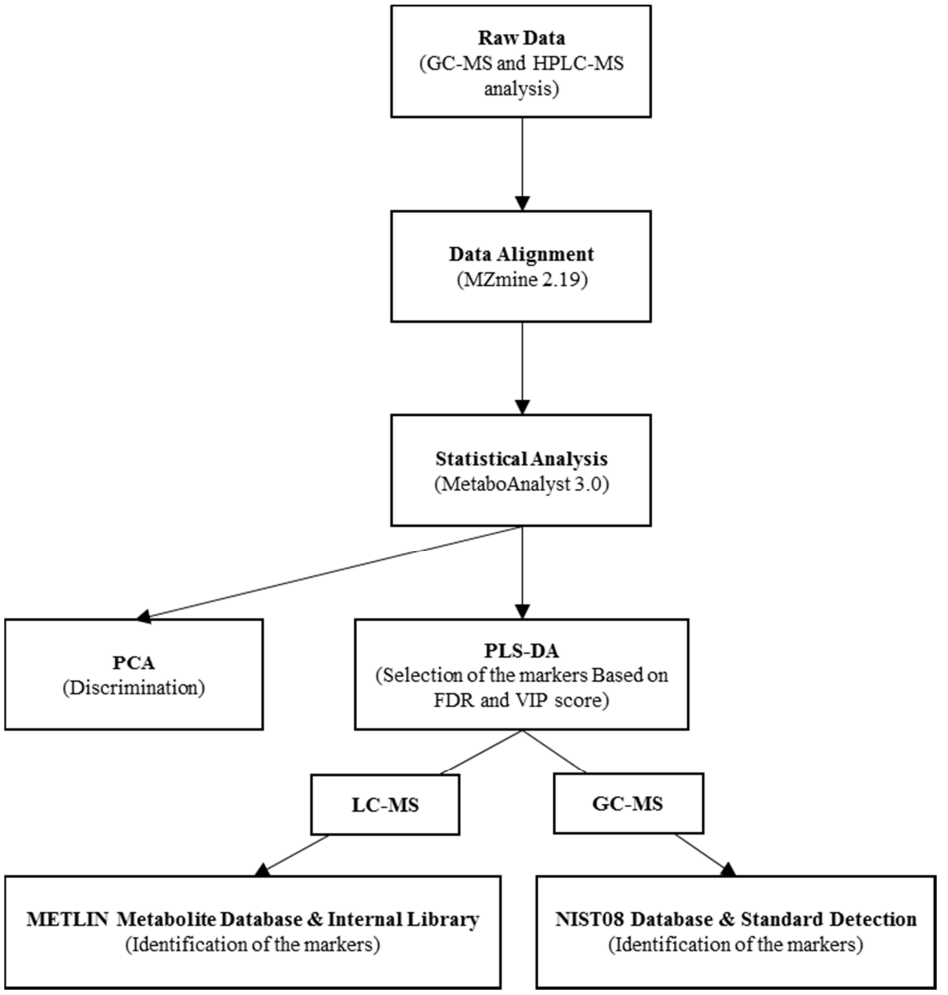


Figure 4. The workflow of data processing.

3. Result and discussion

3. 1. Data processing results

3. 1. 1. Data processing results of GC-MS analysis

PCA, as a statistical analysis used to show the grouping trends, was applied on the GC-MS data of three different cultivars of rice in DOM values of 0, 5, 7, 9 and 11. As shown in Figure 5, the PCA plot depict that rice samples in DOM values of 0, 5 and 7 have a tendency to gather into one group, meanwhile, rice samples in DOM values of 9 and 11 are gathered together as the other group. This appearance illustrate that rice components have no obvious difference within the same group. Moreover, the noticeable differences are also revealed by PCA plot between these two groups. To determine the rice components with remarkable variations among different DOM, which are used as the markers among DOM, PLS-DA model (Figure 6) was applied together with one-way ANOVA. More rigorously, the PLS-DA model was evaluated by the leave-one-out-cross-validation (LOOCV) to test its reliability. According to the R^2 (goodness of fit) value of 0.732 and Q^2 (predictive ability) value of 0.676, which are two parameters used as the results of LOOCV, the discrimination in PLS-DA mode was relatively good. The variable importance in projection (VIP) score and false discovery rate (FDR) in ANOVA analysis were applied to pick out the markers, setting the VIP score greater than 1 and the FDR lower than 0.05 as the criteria of selection. The marker candidates were identified with the NIST08 database and then confirmed using standards. Finally, a total of 10 markers were gained by GC-MS analysis, including sugar group (D-glucose, D-fructose, D-galactose), sugar alcohol group (D-mannitol, D-arabitol, D-glucitol) and amino acid group (L-proline), as well as carboxylic acid group (D-malic acid, oxalic acid, D-gluconic acid). All the markers

were listed in Table 4 and the relatively concentration of the markers in different DOM are listed in Table 5.

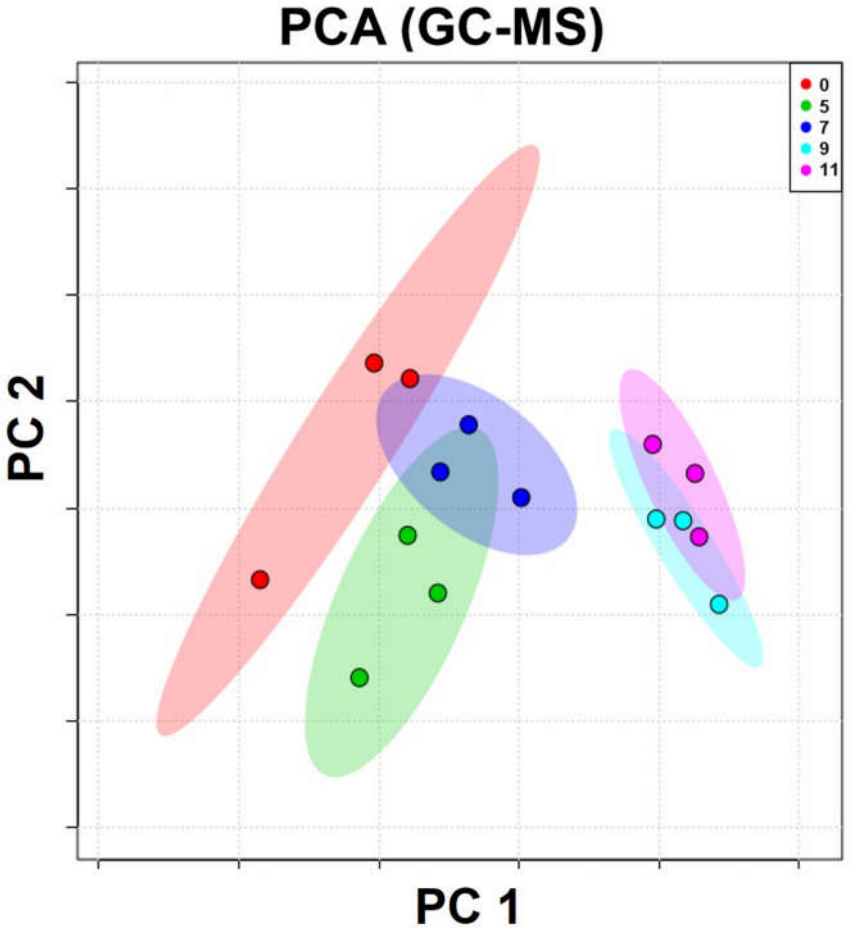


Figure 5. PCA score plot of the rice samples with different DOM in GC-MS analysis.

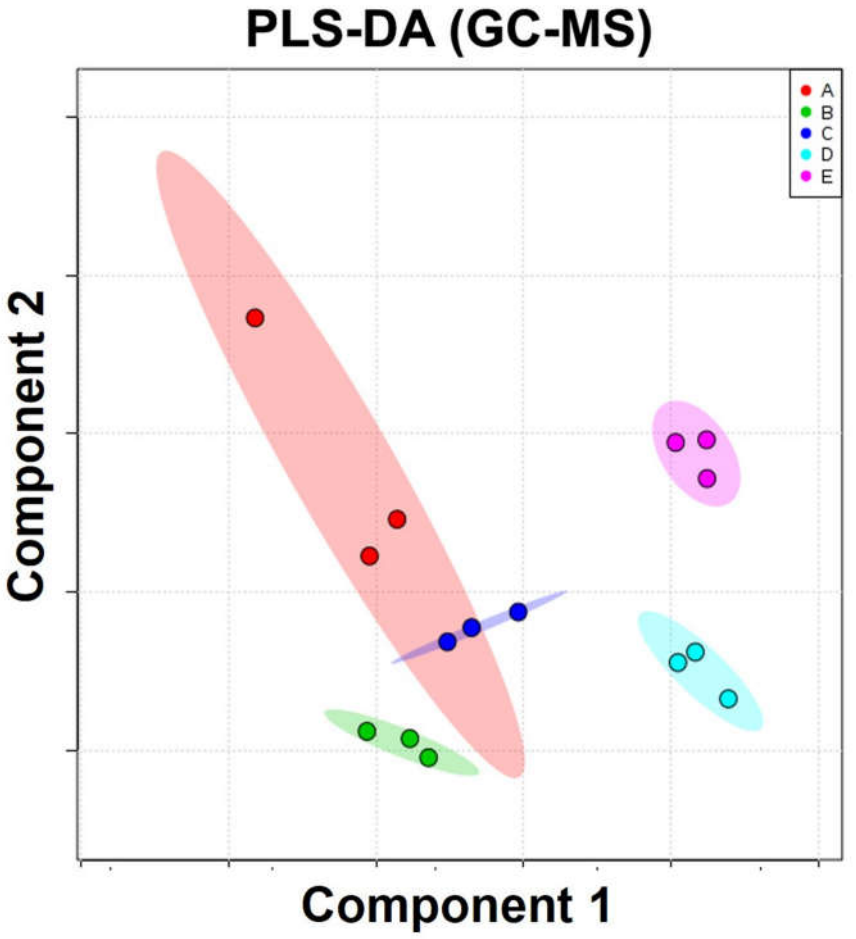


Figure 6. PLS-DA score plot of the rice samples with different DOM in GC-MS analysis.

3. 1. 2. Data processing results of HPLC-MS analysis

Similar as GC-MS analysis, PCA was used with the classification of HPLC-MS data, as shown in Figure 7. In detail, the same grouping tendency with GC-MS analysis still exists. Additionally, PLS-DA was employed for revealing the significant discrimination among samples. Subsequently, the evaluation of PLS-DA model (Figure 8) based on LOOCV showed excellent result with R^2 and Q^2 values of 0.923 and 0.809, respectively. To obtain significant markers, only those who tallied with the criteria of VIP score greater than 1 and FDR lower than 0.05 were selected. The markers were then identified by using the stepwise collision energy MS/MS technique, which confirmed the fragmentation patterns of markers by virtue of the previous study in our laboratory[14]. Following the investigation, we found that all the markers belong to the phospholipids, more specifically, all of them are lysophosphatidylcholines (LysoPC). In summary, as listed in Table 4, the markers of HPLC-MS analysis are LysoPC(14:0), LysoPC(16:0), LysoPC(18:3), LysoPC(18:2), LysoPC(18:1), and LysoPC(18:0). Additionally, the relatively concentration of the markers in different DOM were shown in Table 5.

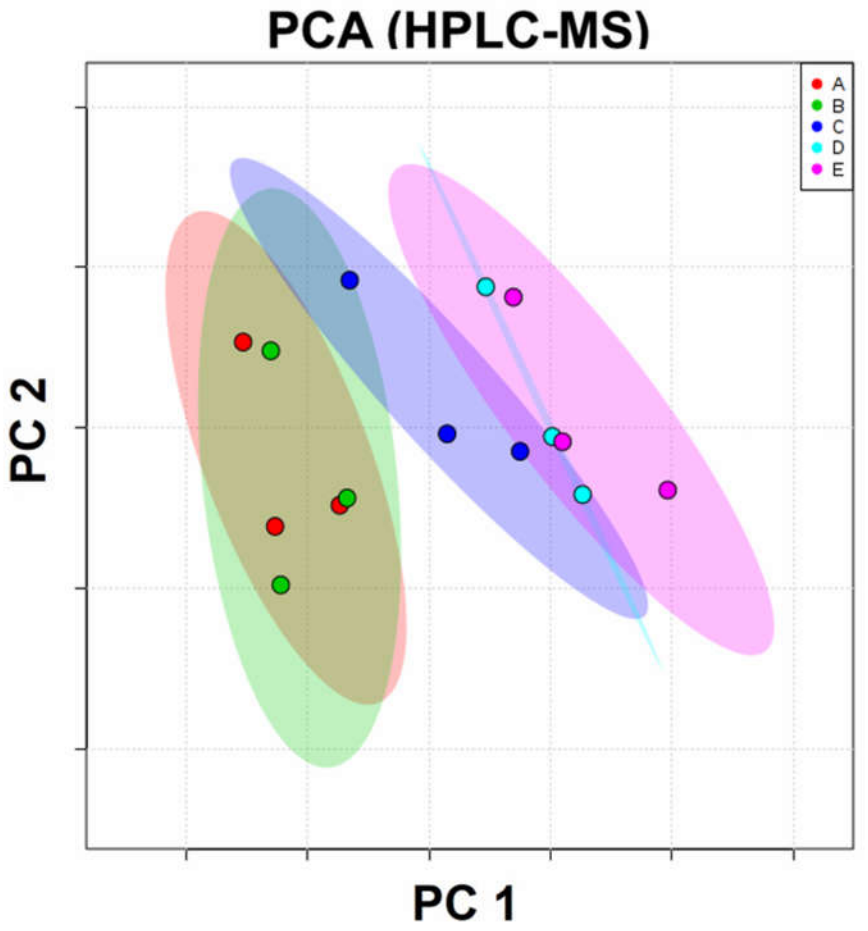


Figure 7. PCA score plot of the rice samples with different DOM in HPLC-MS analysis.

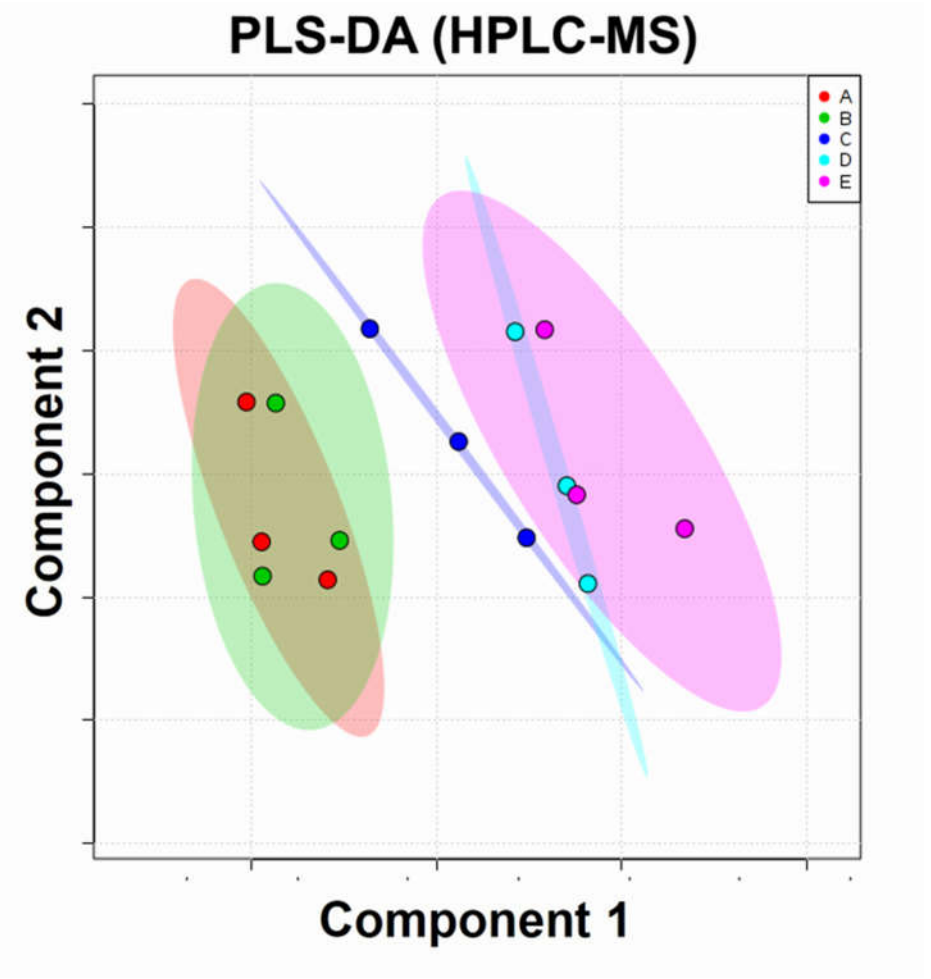


Figure 8. PLS-DA score plot of the rice samples with different DOM in HPLC-MS analysis.

Name of component	Retention time	VIP score	False discovery rate	*p*-value	Instrumental analysis method
Oxalic acid	9.52	2.553	<0.001	<0.001	GC-MS
D-malic acid	17.34	1.984	<0.001	<0.001	GC-MS
L-proline	17.90	1.925	<0.001	<0.001	GC-MS
D-arabitol	21.40	2.384	<0.001	<0.001	GC-MS
D-fructose	24.36	1.114	<0.001	<0.001	GC-MS
D-galactose	24.80	1.249	<0.001	<0.001	GC-MS
D-glucose	25.08	1.131	<0.001	<0.001	GC-MS
D-mannitol	25.28	1.894	<0.001	<0.001	GC-MS
D-glucitol	25.42	2.015	<0.001	<0.001	GC-MS
D-gluconic acid	26.44	3.153	<0.001	<0.001	GC-MS
LysoPC(14:0)	17.20	1.886	<0.001	<0.001	HPLC-MS
LysoPC(18:3)	17.34	1.973	<0.001	<0.001	HPLC-MS
LysoPC(18:2)	18.36	1.734	<0.001	<0.001	HPLC-MS
LysoPC(16:0)	19.87	1.412	<0.001	<0.001	HPLC-MS
LysoPC(18:1)	20.61	1.955	<0.001	<0.001	HPLC-MS
LysoPC(18:0)	23.90	1.242	<0.001	<0.001	HPLC-MS

Table 4. Components with remarkable variations in concentrations.

Compound	Degree of milling				
	0	5	7	9	11
LysoPC(14:0)	1353127	1335999	1479523*	1514284	1524257
LysoPC(18:3)	1339377	1362138	1421423	1459404	1516321
LysoPC(18:2)	3185210	3220310	3372670	3497788**	3520806
LysoPC(16:0)	19548833	19746170	20031591	20401652	20536362
LysoPC(18:1)	13774596	13566995	14037549	14544688	14640271
LysoPC(18:0)	6553068	6623178	6891746	7212533	7301070
D-mannitol	7722306	6901524	6595264	3527745***	3635264
D-arabitol	10962352	9953461	8598961	2425263**	2408529
D-glucitol	8425348	7325143	5852523	1236785	1255291
D-malic acid	8705232	7885636	5364724	319862***	434160*
Oxalic acid	5915263	6652340	8986750	9356730	12649150
D-galactose	2194622	2297618	2476293	1887652*	1611349
D-Fructose	7794619	7879369	9125514	5666615**	4695667
D-glucose	10144437	10706346	10654313	78713710	7419566
L-proline	8313542	8113978	7597150	2874414**	2769371
D-gluconic acid	4955378	4647589	4401043	385521***	513720*

* p-value < 0.05

** p-value < 0.01

*** p-value < 0.001

Table 5. The average peak areas of the markers in different DOM.

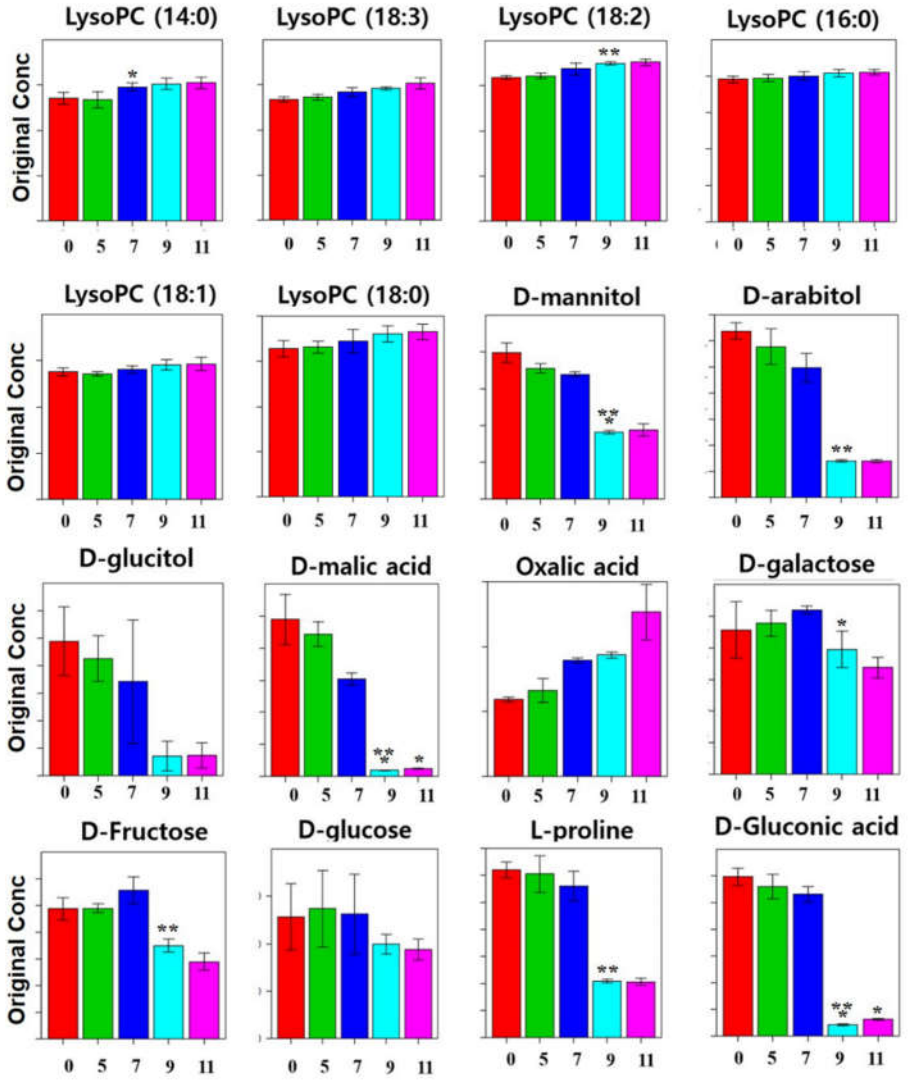
3. 2. Discussion

On the basis of the data processing results of GC-MS and HPLC-MS analysis, the nutritional components of rice with remarkable variations among different DOM and their variation tendency and regularity were revealed in this study. To show them visually, the variation tendency and regularity of markers in GC-MS and HPLC-MS analysis were depicted by comparing the concentration of markers among DOM via box plots. As shown in Figure 9, three different cultivars of rice had the same variation tendency and regularity of markers among DOM. In detail, the concentrations of most markers in GC-MS analysis, except oxalic acid, decreased nonlinearly with the increase of DOM. Among the groups of markers, the decrease tendency and regularity of markers were distinctly different. Specifically, in sugar group, the concentration of markers remained unchanged or even slightly increased with the increase of DOM until reaching DOM of 7 and then reduced in DOM of 9 and 11. At the same time, the concentrations of most markers in sugar alcohol group, amino acid group and carboxylic acid group decreased gradually from DOM of 0 to 7 while dropped sharply from DOM of 9. These variation regularities among DOM demonstrated that the nutritional components are uneven distributed in rice. Additionally, the low milled rice products (until DOM of 7) are abundant in sugars and sugar alcohols.

On the contrary, the concentrations of markers in HPLC-MS analysis increased along with DOM increasing, as shown in Figure 9. The markers, which means phospholipids, maintained at a low concentration state until getting DOM of 7 and had rising tendency in DOM of 9 and 11. Based on variation regularities stated above, the previous investigation was verified that phospholipids mostly exist in rice endosperm[15]. We supposed the reason of increase tendency was that the weight proportion of phospholipids in rice increase with increasing exposed parts of

endosperm caused by milling. Additionally, the high milled rice products, especially rice products in DOM of 11 contains larger proportion of phospholipids.

The benefits of sugar have long been known as the main energy source for the human body in daily life. In addition, most sugar alcohols can provide calories and sweet taste without raising plasma glucose. Nevertheless, the long-term excessive intake of sugars will bring harm to health, such as hyperglycemia and diabetes[16]. On the other side, the health benefits of phospholipids have also been extensively studied before. In brief, considerable research infer that phospholipids may contribute to decreasing cholesterol and cardiovascular risk, improving liver function and producing the anti-inflammatory and anti-cancer effects on human bodies[6, 7, 17].



* p-value < 0.05

** p-value < 0.01

*** p-value < 0.001

Figure 9. Box plots of the marker concentrations in GC-MS and HPLC-MS analysis.

4. Conclusion

In conformity with all the statement in this study, the nutritional value of rice cannot be simply evaluated on brown rice and white rice. Rice in different DOM have their own advantages in health. Three different cultivars of Korean rice have common nutrition features in the same DOM. Specifically, brown rice and the low milled rice (until DOM of 7) are qualified to provide enormous nutrients and calories with less risk of raising plasma glucose to the public by reason that they have more complete structures of rice grain. Particularly, rice products in DOM of 7 have relatively good texture and taste in the low milled rice. However, the high milled rice, especially rice in DOM of 11, contains less sugars but great texture and taste as well as more content of phospholipids which are beneficial to human bodies. So taking these characteristics of rice into consideration, rice production and consumption are necessary to be adjusted according to the different nutritional demands. In conclusion, this study reveals the variation tendency and regularity of nutritional components in rice among different DOM as well as extends and optimizes the evaluations of rice nutritional value. In practical production and consumption of rice, this study is helpful to make rational adjustment to meet a variety of demands. Further investigations would be carried out on the health benefits inferred in this study.

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국문초록

쌀 내에 존재하는 영양성분은 그 중요성으로 인해 오랫동안 과학적 분석의 대상으로 여겨져 왔다. 본 연구의 대상이기도 한 현미 및 백미는 영양적으로 상당한 차이를 나타낸다고 알려져 있으며 일반적으로 현미가 백미에 비해 우수한 영양적 가치를 가지고 있다고 알려져 있다. 그럼에도 불구하고 영양적 가치를 증명하는 포괄적이며 과학적인 연구는 여전히 진행되지 않았다. 특히 단순히 현미, 백미의 두가지 쌀의 성분 비교가 아닌 서로 다른 도정도 (degree of milling, DOM)에 따른 성분의 변화가 연구될 필요성이 있다. 본 연구는 액체 크로마토그래피-질량 분석기 (HPLC-MS) 및 가스 크로마토그래피-질량 분석기 (GC-MS) 기반의 대사체학을 응용하여 0, 5, 7, 9, 11 의 서로 다른 도정도를 가진 쌀을 분석, 다양한 도정도에 따른 성분 변화를 추적하였다. 다양한 도정도간에 유의미한 변화를 보이는 성분을 검출하기 위해 주성분 분석 (principal component analysis, PCA) 및 최소 자승 분석 (partial least squares discriminant analysis, PLS-DA)을 이용하였다. 결론적으로 sugar 및 sugar alcohol 성분 함량이 도정도의 증가에 따라 감소하는 것을 확인할 수 있었다. 반면 phospholipid 의 경우에는 도정도의 증가에 따라 성분의 함량이 증가하는 것을 관찰 할 수 있었다. 이러한 결과는 일반적인 통념과 달리 항상 현미가 영양학적으로 백미보다 우수한 것이 아니며 phospholipid 와 같은 특정 성분의 경우 백미에서 더욱 많은 함량을 섭취할 수 있음을 증명하는 것이다. 도정도에 따른 성분 변화를 관찰한 본 연구를 통해 영양학적인 측면에서의 합리적인 쌀 생산 및 소비에 긍정적인 영향을 기대할 수 있다.

주요어: 쌀 대사체학; 도정도(DOM); 가스 크로마토그래피-질량 분석기 (GC-MS); 액체 크로마토그래피-질량 분석기 (HPLC-MS); Sugar; phospholipid.

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