Chemical Profiling of White, Red, and Black Rice (*Oryza Sativa L.*) During Grain Development Based On LC-QTOF-MS/MS

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Abstract

In this study, the chemical constituents of the three rice varieties were investigated at each of the five stages of grain development using Accurate-Mass Spectrometry (LC-ESI-QTOF-MS/MS). The varieties were: White (Dok Mali105), red (red Dok Mali), and black (Rice Berry) in acidified methanolic extract. Over 80 compounds of the primary and secondary metabolites were tentatively identified. Significant differences in phytochemical content of the rice varieties and maturing stage were clarified. All three varieties used in this study contained the primary metabolites: Sugar, fatty acids, organic acids and amino acids in a free and conjugated form. The secondary metabolites of rice pigments; cyanidin and peonidin glucosides occur in black rice, whereas red rice contains catechin, a compound in procyanidin. The phenolic compounds protocatechuic acid and vanillic acid were found in red and black rice. The secondary metabolites are reported as bioactive and beneficial to health. These phytochemical compounds revealed a distinct grouping of the different rice species on the basis of their metabolite profiles via principal component analysis. This result can be used for selecting a rice variety and stage of grain development at which phytochemical presents relevance to the health benefit and new breeding.

Keywords

Chemical profiling, Grain development, PCA, Rice, *Oryza sativa*

Introduction

Rice (*Oryza sativa L.*) is a staple food of many Asian countries. Thai rice is well known for its unique texture, tenderness, jasmine aroma and distinguished taste. White rice is commonly consumed while red, black, and brown (unpolished white rice) have increased in popularity due to their antioxidant properties and phenolic content. Phenolic compounds are secondary metabolites and are known to have various beneficial physiological effects in humans, such as protection against the development of various cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [1,2]. Black and red rice varieties were likewise demonstrated to have a beneficial anti-oxidative and anti-inflammatory effect in rabbits *in vivo* [3,4]. The cyanidin-3-glucoside and peonidin-3-glucoside in black rice showed antioxidant activities in preventing DNA damage and LDL deterioration *in vitro* and also suppressed the production of nitric oxide in the activated macrophage without introducing cytotoxicity [5].

Three rice varieties; white (Dok Mali105; W), red (red Dok Mali; R) and black (Rice berry; B) were used in this study. Red
rice is a gene mutation from white rice with red color at the pericarp and has a jasmine aroma similar to white rice. The rice berry is a cross-breed between Hom Nil (glutinous black rice) and Dok Mali 105 rice resulting in black color grain and a fragrant smell. The major classes of phenolic compounds present in rice are phenolic acids, flavonoids, anthocyanins and proanthocyanins. While non-pigmented varieties generally contain only phenolic acids, pigmented rice is rich in polyphenolic compounds. In particular, red rice is characterized by the presence of proanthocyanins, whereas black rice contains anthocyanins and proanthocyanins may also be present, depending on the variety [6]. The major phenolic acids found in whole grains are ferulic acid, vanillic acid, caffieic acid, syringic acid and p-coumaric acid [7]. Four different anthocyanins (cyanidin-3-glucoside, peonidin-3-glucoside, cyanidin-3, 5-diglucoside, cyanidin-3-rutinoside) have been identified in black rice [8]. Mavindin and petudinin-3-glycoside have also been found in black rice while the red rice cultivar contains only malvidin [9]. As well as anthocyanins, flavonoids such as; taxifolin, myricetin, isorhamnetin in glycoside form have been detected in Thai black rice bran [10]. Beside this, sucrose esters of hydroxycinnamic acid, ferulic acid and sinapic acid were isolated from methanol extracts of rice bran [11].

Recently, High-performance liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS/MS) is a useful technique in metabolites study. This technique can separated and identify a vast number of chemical compounds present within the plants without purification step. The accuracy of the mass data together with their exact mass, molecular formulas and fragment ions pattern enables good interpretation of results. Thus, structural elucidation can be performed in the absence of the authentic standards.

Several researchers have studied bioactive and phenolic compounds in rice grain and husk during the stages of grain development [12-16], but information on the phytochemical constituents in the rice grain varieties is limited.

The purpose of this study is to characterize phytochemical constituents in five stages of grain development of three rice variety using Accurate-Mass Spectrometry (LC-ESI-QTOF-MS/MS). As well as principal component analysis (PCA) was used for discrimination of rice varieties by means of their phyto-constituents.

### Experimental

#### Chemicals and reagents

Acetonitrile (ACN), methanol (MeOH) and water (LC/MS reagent) were purchased from RCI Lab Scan (Bangkok, Thailand). Formic acid (analytical grade) was obtained from Merck (Darmstadt, Germany). A 0.2 μm Nylon disposable membrane filter was supplied from Vertical Chromatography, Thailand. Other unmarked reagents were of analytical grade.

Reference standard; Cyanidin chloride (PubChem CID: 68247), Peonidin-3-glucoside chloride (PubChem CID: 443654), Cyanidin-3-glucoside (PubChem CID: 92131208), (+)-Catechin (PubChem CID: 9064), were purchased from Fluka, Germany.

### Rice grain production

Three rice varieties; white rice; Dok Mali105 (W), red rice; Red Dok Mali (R) and black rice; Rice berry (B) were used in this study based on the color. Rice plants of each variety was cultivated in Phichit Province, Thailand with the same location and cultivation methods. All rice cultivars were planted in July 2015 and harvested in November 2015. The grain was sampled at 7, 14, 21, 28, and 35 days after the flowering day. The grains were gradually de-hulled by hand, freeze dried, ground with pestle and mortar and kept in plastic bags and then stored at -20 °C for further use [16].

### Acidified methanolic extract of rice samples

The rice powder was defatted and color using hexane, and then the powder was dried in a hot air oven at 50 °C for 5 h. The dried powder (0.5 g/20 ml) was then macerated in acidified methanol (1M HCL:MeOH; 15:85 v/v) for 1 h, and vortexed for 2 min (Vortex genie2, USA) and supernatant was separated by centrifugation at 5000 rpm for 10 min. The residual was re-extracted twice with similar procedures. The pool of supernatant was evaporated to dry at 40 °C under vacuum, after which the extract was freeze dried to make a powder [16]. The extracted powder was weighed and re-dissolved in methanol at a concentration of 15 mg/mL for structural elucidation.

### Standard solutions preparation

Stock solutions of the reference compounds were prepared by dissolving each compound in methanol at concentration 1 mg/mL. The reference compounds mixtures at a concentration of 10 μg/mL were prepared and used for confirmation of the identity.

### Instrumentation LC–ESI-QTOF-MS/MS conditions

Chemical profiling from the acidified methanolic extracts of the rice cultivars with different grain maturity were analyzed by mass spectrometry. The analysis was performed on a 6540 UHD Accurate-Mass-Q-TOF-LC/MS (Agilent Technologies, Palo Alto, CA, USA). The HPLC column was reversed phase Luna C-18, 4.6 x 150 mm, 5 μm (Phenomenex, USA). The mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The conditions for HPLC were as follows: at 0 min 95:5 (A:B v/v) linear gradient to 30:70 (A:B v/v) in 15 min. Post run 5 min for column equilibrium before starting a new injection. Column temperature 35 °C, flow rate 0.5 ml/min, and injection volume 5 μL, were used for all analyses. Mass spectra in the m/z range 100-1200 amu were obtained by both (+) ESI and (-) ESI modes with a 250 ms/spectrum. In the ESI source, nitrogen was used as a nebulizer for drying and as a collision gas. The heated capillary temperature was set to 350 °C and nebulizer pressure to 30 psig. The drying gas flow rate was 10 L/min, and the ion source parameters, voltage for the Vcap, was set at 3500 V, the fragmentor 100 V, skimmer 65 V and octopole RF Peak 750 V. The MS/MS analyses were acquired by auto MS/MS fragmentation where the three most intense mass peaks were fragmented. Collision energy values for MS/MS experiments were fixed at 10 V, 20V and 40 V for each selected...
mass. Accurate mass measurements were obtained by the auto mass calibration method which was performed using an external mass calibration solution (ESI-L Low Concentration Tuning Mix; Agilent calibration solution B). Acquisition of the data was done using the Agilent LC-MS-QTOF Mass Hunter Data Acquisition Software version B.05.01 and the data subsequently analyzed using the Agilent Mass Hunter Qualitative Analysis Software B 06.0 (all software from Agilent Technologies, USA).

**Mass data interpretation**

Chemical identification was performed by comparison of the retention times, mass spectra and fragmentation patterns, with reference compounds, and the published data discovered in our search of the Mass Hunter Metlin metabolite PCD/PCDL database (Agilent Technologies) and the publicly available databases Chemspider (http://www.Chemspider.com), Massbank (http://www.massbank.eu), Lipid Maps Structure Database (http://www.lipidmaps.org). Human Metabolome database (http://www.hmdb.ca). Molecular formula was generated by the Agilent Mass Hunter Qualitative Analysis Software B 06.0. Principal component analysis (PCA) was performed on the statistical software package SIMCA 13.0.3 (Umetrics, Sweden).

**Table 1: Characterization of compounds from acidified methanolic extracts of rice during grain development by LC-ESI-QTOF-MS/MS in positive mode.**

| No. | tR(min) | [M + H] + m/z | MS/MSM/z | Formula | Error (ppm) | Tentative identification | Variety and Stage found |
|-----|---------|---------------|----------|---------|-------------|------------------------|------------------------|
| 2   | 3.01    | 134.0445      | a        | C₆H₇NO₄ | 2.12        | D-Aspartic acid         | W: S1-S5               |
| 6   | 3.06    | 166.0522      | a        | C₇H₆NO₅ | 6.3         | DL-Methionine sulf oxide| W: S4                  |
| 9   | 3.29    | 319.1504      | a        | C₈H₁₂O₅ | 3.74        | Graphinone              | W: S4                  |
| 16  | 4.82    | 234.1341      | 157.0551 | C₉H₁₄NO₂| -2.14       | Hydroxypropionylcarbinil| W: S3                  |
| 46  | 10.95   | 274.0693      | a        | C₁₀H₁₈O₅ | -2.58       | N-feruloylglycine       | W: S4                  |
| 54  | 12.45   | 313.1197      | 105.0478 | C₁₁H₁₀O₅ | 6.24        | Methionyl-Tyrosine      | W: S2                  |
| 5   | 3.06    | 439.1451      | a        | C₁₇H₂₀O₁₃| -1.1        | Pholmiol                | R: S2                  |
| 8   | 3.27    | 116.0708      | C₈H₁₂O₂ | -1.68   | Proline      | R: S1                  |
| 47  | 11.01   | 409.094[M + NH₄] | C₁₁H₁₀NO₅ | 1.25 | Glucoconringin | R: S2                  |
| 25  | 7.37    | 247.1304      | 84.04    | C₁₀H₉O₅ | -6.28       | L-gamma-glutamyl-L-valine| B: S1                  |
| 26  | 7.62    | 306.1569      | 229.07,197.04,169.05,73.03 | C₁₃H₂₃NO₇ | -7.12       | (2R,3S)-5-Ethoxy-2-[[2-methoxynaphoxy]acyllyl amino]-3-methyl-5-oxopentanoic acid | B: S1                  |
| 27  | 7.66    | 190.1086      | 158.08,130.09,98.06 | C₁₂H₁₇NO₃ | -6.39       | Australine              | B: S2-S3               |
| 28  | 7.84    | 227.1403[M + Na]+ | 130.09,70.06 | C₂₄H₂₄O₁₂ | 1.48        | 13-tetradecan-2,4-dien-1-ol | B: S1                  |
| 29  | 7.97    | 196.0981      | 179.07,136.07,91.05 | C₁₀H₁₄NO₅ | -6.53       | Damascenone              | B: S1-S5               |
| 35  | 8.85    | 449.1173      | 287.06   | C₁₃H₁₂O₅ | -21.07      | Cyanidin 3-O-glucoside*  | B: S3-S5               |
| 42  | 9.96    | 287.057       | C₁₃H₁₂O₅ | -6.92   | Cyanidin*     | B: S4-S5               |
| 37  | 9.37    | 463.1269      | 301.07,258.05 | C₁₂H₁₄O₅ | -7.34       | Peonidin glucoside*      | B: S3-S5               |
| 44  | 10.32   | 222.0769      | 162.05,144.04,89.044 | C₁₁H₁₄NO₄ | -3.67       | 6-Hydroxyindolelactate  | B: S5                  |
| 49  | 11.32   | 183.0637[M + Na]+ | a        | C₁₃H₁₆O₄ | 0.22        | 3,3-Dimethyl-glutaric acid | B: S1                  |

**Results and Discussion**

In this study, the molecular formula was established by high-accuracy quasi-molecular ions such as [M-H] ; [M + Cl], [M + HCOO], and [M + Na]; with an error mass of 15 ppm and fractional isotope abundance, and the most rational molecular formula was searched in the various chemical data bases. The mass fragmentations were used to further confirm the chemical structure. The chloride adduct [M + Cl] occurred due to the extraction solvent containing hydrochloric acid (HCl).

**Identification of chemical constituents in rice; Primary metabolites**

The mass data in the positive and negative modes showed m/z as an even number which implies that the nitrogen contained at least one molecule. Thirty-eight compounds were identified as amino acids in free and conjugated form, but which varied depending on rice varieties and stage of grain development. The sugars: Glucose and sucrose were also found, as were fatty acids conjugated with amino acids. The basis of the proposed identifications is shown in (Table 1) [positive mode (+ ESI)] and (Table 2) [negative mode (-ESI)]. The data in table is category in each variety of rice, two com-
| No. | Ret.  | m/z | M/CH_{3}OH + H | Exact M.Wt. | Formula | Mass Error % | Description |
|-----|-------|-----|---------------|-------------|---------|--------------|-------------|
| 56  | 12.72 | 252.0885 | a | C_{12}H_{13}NO_5 | -7.37 | 2-[(3- Carboxypropyl) carbamoyl] benzoic acid | B: S1 |
| 57  | 13.12 | 271.1171[M + CH3OH + H] | 239.08,129.03 | C_{12}H_{14}O_5 | 1.9 | Trans-2,3,4- trimethoxycinnamate | B: S1,S2 |
| 7   | 3.18  | 236.149 | 104.11 | C_{10}H_{12}N_3 | 1.06 | 2,5-Anhydro-1-deoxy-1-(((2R)-1-hydroxy-2-butanyl) amino)-D-glucitol | W: S1,S2,S4; B: S3,S4 |
| 19  | 5.45  | 182.081 | 91.05 | C_{12}H_{12}O_5 | 0.94 | N-Hydroxy-L-phenylalanine | W: S1-S3; B: S1-S5 |
| 24  | 7.35  | 216.1244 | 157.05,113.03,85.03 | C_{12}H_{14}N_2 | -6.32 | Diethyl[[dimethylamino] methylene] malonate | W: S1,B: S1 |
| 10  | 3.35  | 148.0604 | 102.05 | C_{13}H_{16}O_4 | 0.23 | Glutamate | W: S1-S5; R: S2 |
| 14  | 3.54  | 176.0916 | 117.05,85.03 | C_{12}H_{14}O_5 | 0.74 | N-Carboxyethyl-g- aminobutyric acid | W: S3,S5; R: S1-S3 |
| 18  | 5.33  | 305.0845[M + Na]+ | C_{10}H_{18}O_9 | -0.64 | Xylobiose | W: S1-S4; R: S3 |
| 33  | 8.8   | 265.1601 | 177.05,145.03, 117.03,89.04 | C_{12}H_{16}O_8 | -5.33 | (E)-3-(4-isopropylphenyl)-1-(p-tolyl)pro-2-en-1-one | W: S1-S5; R: S2 |
| 51  | 11.43 | 495.2429[2M + H]+ | C_{18}H_{32}O_{16} | -1.03 | 6-alpha-Maltosylglucose | W: S3-S5; R: S1; B: S1-S5 |
| 40  | 9.96  | 325.0913 | a | C_{12}H_{16}O_8 | 1.52 | (3,4-Diacetoxyphenyl) methylene diacetate | R: S3,S5; B: S2 |
| 43  | 10.07 | 419.1186 | 345.08,243.05,86.09 | C_{12}H_{16}O_{12} | -0.47 | 1-(3,4-Diacetoxy-5-oxotetrahydro-2-furanyl)-1,2,3-propanetriyl triacetate | R: S5; B: S2 |
| 1   | 2.94  | 266.1605 | 104.11,60.08 | C_{11}H_{16}O_8 | -2.58 | 5-Aminopentyl-a-D- mannopyranoside | W: S1-S4; R: S1,S4,B: S1-S5 |
| 3   | 3.1   | 365.1054[M + Na]+ | C_{12}H_{16}O_{12} | 0.09 | Sucrose | W: S3-S5; R: S1-S5; B: S1-S2, S5 |
| 4   | 3.03  | 527.1588[M + Na]+ | a | C_{12}H_{16}O_{12} | -1.03 | 6-alpha-Maltosylglucose | W: S3-S5; R: S1; B: S5 |
| 11  | 3.41  | 136.0622 | 119.03,65.01 | C_{11}H_{16}N_3 | -3.15 | Adenine | W: S1-S5; R: S1-S3,B: S1-S5 |
| 12  | 3.47  | 333.1665 | 128.08 | C_{12}H_{16}O_5 | 2.24 | Methyl 8-[2-(2-formylvinyl)-3-hydroxy-5-oxo- cyclopentyl]-octanoate | W: S1-S4; R: S4,B: S1-S5,S3-S5 |
| 13  | 3.5   | 152.0568 | 135.03,110.03 | C_{11}H_{16}O_5 | -0.75 | 8-Hydroxyadenine | W: S1; R:S1,B: S2-S3 |
| 15  | 3.76  | 162.0761 | 102.05,84.04,56.05 | C_{11}H_{16}O_4 | -0.1 | N-Methylglutamic acid | W: S1-S5;R: S1-S5,B: S1-S5 |
| 17  | 4.96  | 379.123 | 217.07 | C_{12}H_{16}O_11 | 1.29 | 1-C-(Acetoxyethyl)-2,3,4- tri-O-acetylhexopyranoside | W: S1-S5; R: S1-S5,B: S1-S5 |
| 20  | 5.64  | 176.0927 | 130.2 | C_{11}H_{16}O_4 | -5.52 | Diethyl aminomalonate | W: S1-S5; R: S1-S5,B: S1-S5 |
| 21  | 6.19  | 160.1082 | 101.05,59.05 | C_{11}H_{16}N_2 | -0.92 | delta-Guanidinovaleric acid | W: S3-S4; R: S3-S5,B: S1-S5 |
| 22  | 6.25  | 132.1019 | 86.09 | C_{11}H_{16}O_2 | 0.04 | L-Norleucine | W: S1-S5; R: S3-S4;B: S1-S5 |
| 23  | 7.27  | 212.1296 | 145.05,127.04,85.03 | C_{11}H_{16}N_2 | -1.79 | Isoprenaline | W: S4; R: S3-S5; B:S1 |
| 30  | 8.07  | 166.0872 | 120.08,77.04 | C_{11}H_{16}N_2 | -5.72 | Phenylalanine | W: S1-S5; R: S1-S4,B: S1-S5 |
| 31  | 8.43  | 144.0663 | 84.04 | C_{11}H_{16}O_3 | -5.42 | N-Acrylglycine methyl ester | W: S1-S5; R: S1-S4,B: S1-S5 |

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Table 2: Characterization of compounds from acidified methanolic extracts of rice during grain development by LC-ESI-QTOF-MS/MS in negative mode.

| No. | tR (min) | [M-H]-m/z | MS/MS m/z | Formula | Error (ppm) | Tentative identification | Rice variety and Stage found |
|-----|----------|-----------|-----------|---------|-------------|--------------------------|-----------------------------|
| 19  | 8.65     | 359.0743  |           | a       | C₁₀H₁₅NO₂   | 8.16                     | 5',2',3'-Trihydroxy-3,6,7-trimethoxyflavone | W: S1-S5; R: S2-S5; B: S1-S5 |
| 21  | 8.97     | 439.1918  |           | 403.22,359.19,329.18,297.15,186.12,127.05 | C₁₉H₁₀O₅ | -5.71                  | 1-alpha-O-Methylquassin     | W: S4-S5                      |
| 25  | 9.38     | 176.0369  |           | a       | C₆H₁₀NO₅S  | 10.09                   | N-Formylmethionine          | W: S4                         |
| 28  | 9.73     | 325.0497  |           | 289.07,245.07 | C₆H₄O₄ | -3.92                  | Catechin*                  | R: S2-S3                      |
| 2   | 2.99     | 104.0348  |           | 74.02 | C₆H₄NO₄  | 4.97                    | D-Serine                   | B: S1-S3                      |
| 3   | 3.01     | 225.0599  |           | 179.05,161.04 | C₅H₄O₅ | 1.73                    | Hexose                     | B: S1-S3,S5                   |
| 15  | 7.22     | 147.0317  |           | 129.01,85.02 | C₅H₄O₃ | -12.18                  | (S)-2-Hydroxyglutarate     | B: S1                         |
| 17  | 8.04     | 164.0733  |           | a       | C₆H₄NO₄  | -9.68                   | Phenylalanine              | B: S2                         |
| 20  | 8.84     | 465.1006  |           | 285.04,329.08 | C₁₉H₁₅O₈ | -19.85                  | Cyanidin glucoside*        | B: S4-S5                      |
| 8.85 | 447.089[M-H]- | 284.03,256.03 |           |         | C₁₉H₁₅O₈ | -19.85                  | Cyanidin glucoside*        | B: S4-S5                      |
| 24  | 9.36     | 479.1143[M+Na]+ | 355.06,299.05,284.03,149.02 | C₁₉H₁₅O₈ | 10.85                  | Peonidin glucoside*       | B: S4-S5                      |
| 30  | 11.68    | 463.083[M-H]- | 301.03,257.04,109.02 | C₁₉H₁₅O₈ | 11.23                  | Tricetin-7-glucoside      | B: S5                         |
| 5   | 3.16     | 171.0081  |           | 78.95 | C₅H₄O₅P  | -9.95                   | Glycerol-3-phosphate       | R: S2,S4,S5; B: S1,S3,S5      |

W = White Rice, R = Red Rice, B = Black Rice, S = Stage, a = Fragmentation was not achieved.
Secondary metabolites are named as bioactive compounds. In black rice at stages S5, 359.0987 [M+Cl]- (9.3 min) was observed. These two mass spectra had ion fragments of m/z 287 and 301, respectively, suggesting the loss of one glucose moiety (m/z 162). These two compounds are cyanidin-3-glucoside and peonidin-3-glucoside which we identified by comparison with authentic compounds. Cyanidin was also found in black rice at stages S1, 2, 3, 4, 5. Other phenolic compounds found included trans-4-hydroxy-3-methoxycinnamate, 4,2',4'-Trihydroxydihydrochalcone (B: S1), Protocatechuic acid (R: S4-S5, B: S2-S5), Catechin (R: S2, S3), p-Hydroxybenzoic acid, Tricetin-7-glucoside (B: S5), Vanillyl acid (R: S2-S5, B: S2-S5), 3,3',4,5'-Tetrahydroxy-trans-stilbene, and 5,8,4'-Trihydroxy-3,6,7-trimethoxy flavone. In this experiment, red rice does not contain anthocyanin pigments, which is in agreement with Pengkumsri, et al. [17] and also with Kim, et al. [18] who concluded also that red rice does not contain anthocyanin pigments but also found that red rice contains the procyanidin called catechin, and 5,8,4'-Trihydroxy-3,6,7-trimethoxy flavone. We found that black rice contains 5,8,4'-Trihydroxy-3,6,7-trimethoxy flavone and 3,3',4,5'-Tetrahydroxy-trans-stilbene.

Identification of chemical constituents in rice; Secondary metabolites

Secondary metabolites are named as bioactive compounds. In black rice at stages 3-5, the m/z 449 [M]+ (8.8 min) and m/z 463 [M]+ (9.3 min) were observed. These two mass spectra had ion fragments of m/z 287 and 301, respectively, suggesting the loss of one glucose moiety (m/z 162). These two compounds are cyanidin-3-glucoside and peonidin-3-glucoside which we identified by comparison with authentic compounds. Cyanidin was also found in black rice at stages (S) 4, 5. Other phenolic compounds found included trans-4-hydroxy-3-methoxycinnamate, 4,2',4'-Trihydroxydihydrochalcone (B: S1), Protocatechuic acid (R: S4-S5, B: S2-S5), Catechin (R: S2, S3), p-Hydroxybenzoic acid, Tricetin-7-glucoside (B: S5), Vanillyl acid (R: S2-S5, B: S2-S5), 3,3',4,5'-Tetrahydroxy-trans-stilbene, and 5,8,4'-Trihydroxy-3,6,7-trimethoxy flavone. In this experiment, red rice does not contain anthocyanin pigments, which is in agreement with Pengkumsri, et al. [17] and also with Kim, et al. [18] who concluded also that red rice does not contain anthocyanin pigments but also found that red rice contains the procyanidin called catechin, which we found as well. The chromatogram and proposed identification are shown in (Figure 1) and (Table 1 and Table 2).

Discrimination of white, red and black rice grain by LC-MS fingerprint analysis and chemometrics
Remarkable differences were observed in the contents of the compounds in all samples. PCA model was used for discrimination and classification of samples according to their type of the rice and stages of grain development. In this PCA study, retention times and peak intensities of each chromatogram in both negative and positive modes were bucketed at every 0.004 min using the SIMCA Software. All obtained data were then analyzed for giving scores plots of principle component 1 (PC1) versus principle component 2 (PC2) (Figure 2). The PCA scores plot of the data from negative ionization pre-
Figure 2: Principal Component Analysis (PCA) of phytochemical data of rice three varieties at five stages of grain development (S1-S5). A) Negative mode; B) Positive mode. PCA was plotted between retention time and peak intensity.

Conclusions

This study describes a comprehensive investigation of phyto-constituents in three rice varieties with different phenotypic background and stages of grain development. The complex development of phytochemicals in rice varieties which we confidently suggest will be of significant benefit to the agri-food industry, informing the future work of developing new rice varieties with enhanced health promoting properties.
phytochemicals. In addition to phytochemical information, we provide an important framework for quality control analysis and the determination of varieties by applying chemical profiling in our analysis. The established LC-ESI–QTOF-MS/MS approach is shown to be a suitable and powerful for the qualitative analysis of the phytochemicals in rice.

Credit Author Ship Contribution Statement

Nitra Nuengchamnong: Conceptualization, Methodology, Visualization, Software, Writing-Review & Editing. Tongchai SaeSong: Data Curation, Software. Paradon Ngamdee: Methodology. Sudarat Jiamyangyuen: Project Administration, Resources, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

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