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Chemical profiles of three varieties of germinated rice based on LC-MS and their antioxidant activity

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Abstract

In this study, chemical profiles in different germinated rice extracts (GREs) using different solvent extraction ratio were investigated. Three varieties of germinated rice (GR), including germinated white rice (GWR), germinated black rice (GBR) and germinated red rice (GRR) were extracted using 70 and 100% ethanol (v/v). Both extracts were characterized for their chemical profiles using liquid chromatography-electrospray ionization–quadrupole–time–of–flight mass spectrometry (LC–ESI–Q–TOF–MS). The content of γ–aminobutyric acid (GABA), total phenolic content (TPC), and antioxidant activities were also determined. The chemical profiles of GREs are composed of organic acids, amino acids, vitamins, flavonoids, and phenolic compounds. The GABA content of all rice varieties presented the same pattern in both ethanolic extracts. The TPC of GRE extracted by 70% ethanol (v/v) showed significant higher amount than that in the 100% v/v ethanolic extract (p<0.05). The highest TPC was obtained from GBR, followed by GRR and GWR, respectively (p<0.05). The antioxidant activity from three assays, including DPPH, ABTS, and FRAP showed higher activities in the 100% v/v ethanolic extracts than their 70% v/v counterparts (p< 0.05). The phenolic content showed a low positive Pearson correlation with antioxidant activities, however, the strong positive Pearson's correlation coefficients were observed among these activities (r = 0.846-0.935). The results suggested that the GR was composed of potential bioactive compounds such as GABA and other phytochemical contents possessing high antioxidant bioactivity which can be used as functional food or as part of nutraceutical products.

Keywords: Germinated rice extract, Chemical profiling, Antioxidant activity, LC–ESI–Q–TOF–MS, γ-aminobutyric acid

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1. Introduction

Consumption of food rich in nutritional components and bioactive compounds is promoted as benefits for human health (Abuajah *et al*., 2015). Many extraction techniques have been established to extract polyphenols from plants since no single universal extraction method can be applied because of the complexity of phenolic compounds and their interaction with other bioactive components in the plant matrix (Liyana-Pathirana and Shahidi, 2005). In addition, several factors, which can affect the extraction efficiency, can cause a variation in antioxidant content (Arabshahi-D *et al*., 2007). For example, extraction of antioxidants is largely depended on solvents. Ethanol has been known as an effective solvent for polyphenol extraction and is desirable due to its less toxicity. For instance, aqueous ethanol was reported as the most efficient and suitable solvent for the extraction of phenolic compounds from wheat (Liyana-Pathirana and Shahidi, 2005).

Germination of cereal grains is responsible for many biochemical changes within the grains. During the germination, water activates the hydrolytic enzymes that hydrolyze the large molecular constituents, such as proteins, starch, and other polysaccharides to small molecules, resulting in the increase of amino acids, peptides, sugars, and oligosaccharides in germinated seeds. Germinated rice (GR) is recently considered as functional food, because it does not only contain basic nutritional components, including vitamins, minerals, dietary fibers, and essential amino acids, but also accumulates a considerable amount of biochemical compounds, such as linoleic acid, tocotrienols, and γ-oryzanol (Moongngarm and Saetung, 2010). In addition, γ-aminobutyric acid (GABA) is one of the major components that shows significantly increased in GR (Kim *et al*., 2012; Moongngarm and Saetung, 2010). Furthermore, other bioactive components, such as ferulic acid and sinapinic acid, were also found in the GR (Tian *et al*., 2004).

Some recent studies have profiled the nutritional and bioactive metabolites in the GR using gas chromatography coupled with mass spectrometry and nuclear magnetic resonance (NMR) analysis (Ding *et al*., 2018; Pramai *et al*., 2018). The compounds identified by NMR, were α-linolenic acid, γ-oryzanol, α-tocopherol, 3-hydroxybutyric acid, fumaric acid, fatty acids, vanillic acid, which were reported to have a strong positive correlation with the DPPH radical scavenging activity in germinated rice extracts (GREs) (Pramai *et al*., 2018). In addition, the advanced analytical techniques included a combination of gas chromatography or liquid chromatography with mass spectrometry (MS) were applied to elucidate the metabolites from rice (Kim *et al*., 2013; Yang *et al*., 2014), however, limited studies are done on GR. Therefore, further studies are required to explore the biochemical components presented in GR.

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Liquid chromatography-electrospray ionization–quadrupole–time–of–flight mass spectrometry (LC–ESI–Q–TOF–MS) allows for the identification of many bioactive compounds due to its sensitivity and a wide range of compound detection (Yang *et al*., 2014). In this study, the chemical profiles of three varieties of GR, which extracted by different ethanol/water ratio were characterized using LC–ESI–Q–TOF–MS analysis. The GREs were further evaluated for their GABA content, total phenolic content (TPC), and the antioxidant activity that was determined through different antioxidant assays, including 1,1–diphenyl–2picrylhydrazyl (DPPH) radical scavenging ability, ABTS radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay. The obtained information might be helpful to reveal the bioactive components in the GR and may provide guidance for its application in food supplements or nutraceutical products.

2. Materials and Methods

2.1 Chemicals and reagents

Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), DPPH, 2,2'–azino–bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6–tripyridyl–s–triazine (TPTZ), gallic acid, 6–hydroxy–2,5,7,8–tetramethylchroman–2–carboxylic acid (Trolox), GABA, and 2–hydroxy–1–naphthaldehyde (HN) were purchased from Sigma–Aldrich (Steinheim, Germany). Absolute ethanol and all HPLC solvents were purchased from Merck Millipore International (Darmstadt, Germany).

2.2 Plant materials and germinated rice cultivation

Three paddy rice varieties included *Oryza sativa* L. cv. Hom Deang Sukhothai 1 (red rice; RR), *O. sativa* L. cv. Hom Dam Sukhothai 2 (black rice; BR), and *O. sativa* L. cv. Hom Mali 105 (white rice; WR) were provided by the Organic Agriculture Project, Sukhothai Airport, Thailand.

Five hundred grams of paddy rice samples were soaked in 2.5 L of water for 24 h at room temperature and the water was changed every 8 h. The samples were placed on double layers of cotton cloth that was stretched on six plastic containers (28 × 38 × 10 cm) contained water at 6 cm high from the bottom. The samples were allowed to germinate at room temperature and randomly collected from each container after 10 days of germination. The dried form of germinated paddy rice was prepared using a hot air oven at 45 °C for 24 h. The dried samples were ground to a fine powder using laboratory grinder (Waring Commercial, MX1100XTX Xtreme). The powdered samples were packed in polyethylene bags and stored at 4 °C prior to use for sample extraction.

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2.3 Extraction

The powdered GR (5 g) was mixed with 50 mL solvent (70 and 100% ethanol, v/v). Extraction was performed on an ultrasonic bath (Kudos, Shanghai, China) at room temperature for 1 h. The mixtures were then filtered through the Whatman paper (No.1) and the residues were re-extracted with the same solvent under the same conditions. The filtrate from each extract was combined and then evaporated under vacuum at 40°C to remove the solvents using a rotary evaporator (Buchi 23022A010). The obtained GREs were kept at −20 °C for further analysis.

2.4 ESI−Q−TOF−MS analysis and identification

Mass spectra were acquired using G6540B MS Q−TOF (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 1260 infinity high-performance liquid chromatography (HPLC) instrument (Agilent, Waldbonn, Germany) and an electrospray ionization (ESI) source. The instrument was equipped with a G1312B binary pump and G1367E HiP sampler. The separation was carried out with Phenomenex Luna C−18(2) column (5 µm, 150×4.6 mm) (Phenomenex, CA, USA) with gradient mobile phase comprising of water (Solvent A) and acetonitrile (Solvent B), both of which contained 0.1% v/v formic acid. The linear gradient was performed from 5 to 95% (v/v) of Solvent B over 20 min at 35 ºC with a flow rate of 0.5 mL/min. The injection volume in the system was 5 µL. Other analytical parameters were set using negative ion mode with spectra obtained over a mass range of m/z 100-1,200 amu. The condition of ESI-MS parameters was proceeded as capillary voltage at 3,500 V; dry gas temperature at 350 °C; dry gas flow at 10 L/min; nebulizer pressure at 30 psi and scan rate was at 4 spectra/sec.

The identification for chemical components of GREs was conducted by the comparison of retention time, mass data, and fragmentation patterns with authentic compounds, literature data, library search of the Mass Hunter METLIN metabolite database (Agilent Technologies), and public database (Human Metabolome Database: HMDB).

2.5 Analysis of GABA content

The GABA content was measured with high-performance liquid chromatography (HPLC) based on the modified method of Jannoey *et al*. (2010). GREs (1.0 mL) was derivatized by mixing with 0.5 mL of borax buffer (pH 8) and 0.5 mL of HN (0.3%, w/v in methanol) (Khuhawar and Rajper, 2003). The mixture was heated at 80 °C for 10 min and then allowed to be cooled down. The solution was filtered through a 0.45 µm membrane filter and analyzed by HPLC. To perform the analysis, the reversed-phase HPLC (Model Jasco LC Net II/ADC, JASCO International, Tokyo, Japan) system was equipped with a Jusco PU-2089 pump, a Jusco CO−2065 Plus column oven and a UV/VIS detector (Jasco UV-2077 Plus). The

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operation was controlled by ChromNAV software and GABA was separated by a Waters Xterra C18 column (4.6×150 mm, 5 µm). The mobile phase was consisted of acetonitrile (A) and 0.1% v/v formic acid in water (B) at a flow rate of 1.0 mL/min. Gradient elution was started from 65 to 60% v/v solvent B at 0 to 5 min, 60 to 45% v/v solvent B at 5 to 10 min and 45 to 65% v/v solvent B at 10 to 20 min. The column temperature was set at 30 °C and the wavelength was at 330 nm. The GABA content was detected and quantified using an external standard method.

2.6 Total phenolic content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu assay as described by Zhang *et al*. (2006). An aliquot of 20 µL of each GRE and gallic acid standard solutions were loaded on a 96–well micro-plate. An aliquot of 100 µL of fresh Folin–Ciocalteu reagent was added and well mixed with sample and standard solutions. After 5 min, 80 µL of 7.5% (w/v) sodium carbonate was added and then the sample mixtures were left in the dark at room temperature for 2 h. The absorbance at 765 nm was taken using a SPECTRAmax PLUS microplate reader (Molecular Devices, San Jose, CA, USA). The TPC result was expressed as mg gallic acid equivalent (GAE)/g extract.

2.7 DPPH radical scavenging assay

The DPPH radical scavenging ability of various GREs was performed according to a previous report described by Pellati et al. (2004) with a slight modification. Aliquots (100 µL) of diluted GREs were added to 2.9 mL of 0.1 mM DPPH in methanol. The mixtures were vortexed and left to stand in the dark for 30 min at ambient temperature. The absorbance of each reaction mixture was measured at 517 nm against a blank using a UV–Visible spectrophotometer (Genesys 20, Thermo Scientific, USA). The DPPH radical scavenging ability was calculated as %inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$. A calibration curve was plotted with % inhibition versus concentration of trolox standard antioxidant in a range of 0-400 µM. The antioxidant capacity of GREs was expressed as mmol of trolox equivalent/g extract.

2.8 ABTS radical scavenging assay

The ABTS radical scavenging assay was determined based on the method reported by Pellegrini *et al*. (2003) with slight modification. A stable stock solution $ABTS^{\bullet+}$ cation radical was generated by the reaction between 5 mL of aqueous ABTS solution (7 mM) and 88 µL of potassium persulfate solution (140 mM). The reaction mixture was stored in the dark at ambient temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was diluted by ethanol to an absorbance of 0.70 (±0.02) at 734 nm. An amount of 100 µL of GREs and trolox standard solution was reacted with 2.90 mL of $ABTS^{\bullet+}$ solution. The absorbance of each mixture and control was measured at 734 nm after reaction time for 1 min using a spectrophotometer

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(Genesys 20, Thermo Scientific, USA). The ABTS radical scavenging ability was calculated as %inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$. A concentration of trolox standard solution in a range of 0–400 mM was used for the calibration curve. The results of ABTS radical scavenging assay were expressed as mmol of trolox equivalent/ g extract.

2.9 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined following the slightly modified method of Benzie and Strain (1999). The FRAP reagent was daily prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at a ratio of 10:1:1 (v/v/v). One hundred microliters of GREs and trolox standard solution were mixed with 2.90 mL of FRAP reagent. The mixtures were incubated at 37 °C for 30 min before the absorbance was measured against blank at 595 nm using a spectrophotometer (Genesys 20, Thermo Scientific, USA). A calibration curve was performed over the range of 0–500 mM concentration of trolox. The FRAP values were expressed as mmol of trolox equivalent / g extract.

2.10 Statistical analysis

Results and values were expressed as mean and standard deviation (n=6). Statistical analyses were analyzed using SPSS statistics 17.0 (SPSS Inc., Chicago, IL, USA). Significant differences in means were determined using analysis of variance (ANOVA) and the significance level was set at p=0.05. Duncan's new multiple range test was performed to determine the multiple mean comparisons within the sample set. Correlations between GABA content, TPC, and antioxidant capacities were evaluated using Pearson's correlation coefficient test.

3. Results and Discussion

3.1 Phytochemical characterization

The total ion chromatograms (TIC) of three GREs in the negative mode are shown in Fig 1. The patterns of chromatographic profiles of the extracts with same ethanol concentration were similar, but the chemical components among sample varieties were slightly different. Forty-three peaks were detected and 33 compounds were identified based on the LC–MS data, following the confirmation by comparison to reported data, library search, and authentic compounds (Table 1).

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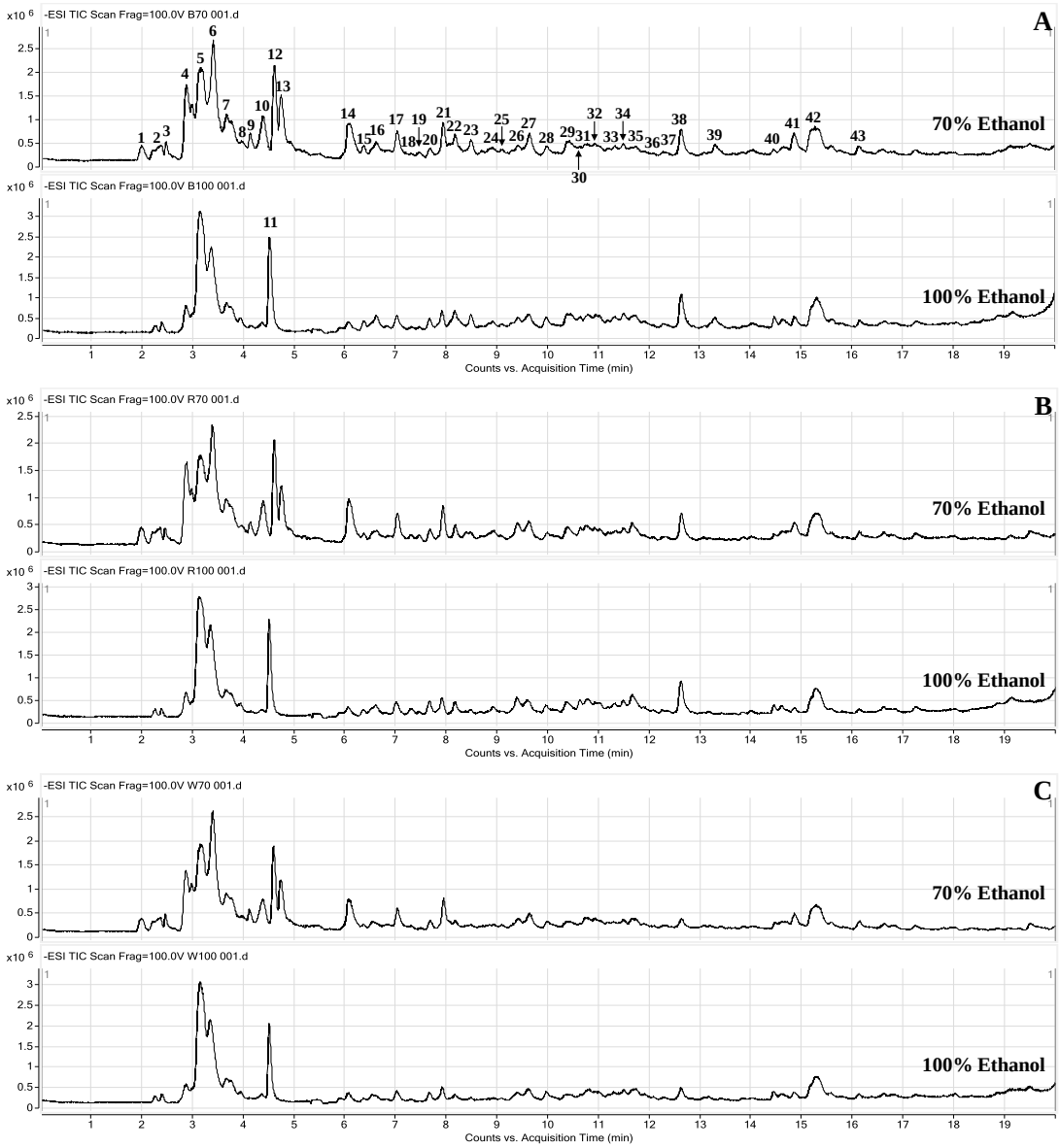

Figure 1 Total ion current (TIC) chromatograms of 3 mg/mL of GBR (A), GRR (B), and GWR (C). Monitored in electrospray ionization (ESI) negative mode. The peak numbers and compounds identification were summarized in Table 1

The identified chemical components in GREs were suggested as sugars, small organic acids, amino acids, vitamins, flavonoid derivatives, and phenolic acids. The results showed that more identified compounds were observed in the GBR extracts of both 70 and 100% v/v ethanol extraction, followed by those of GRR and GWR, respectively. In term of extraction solvent ratio, the extracts from 70% v/v ethanol extraction displayed more peaks than that of 100% ethanol (v/v) for all the GR varieties. This may be attributed to the chemical components, such as carbohydrates and proteins that more extractable with aqueous solution than in pure organic solvent (Zhu *et al*., 2011). As a significant amino acid in the GR, GABA (3)

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was detected at t_R 2.401 min with $[M-H]^-$ ion at *m/z* 102.0562 corresponding with a database search. Peak 4 with *m/z* 131.0454 $[M-H]^-$ and a fragment ion $[M-H]^-$ at *m/z* at 113.1689 was identified as D-asparagine (Piraud *et al*., 2003). Peak 17 and 21 with *m/z* $[M-H]^-$ at 164.0725 and 203.0829 were proposed as two amino acids for phenylalanine and tryptophan, respectively, based on their molecular formula and fragment ions consent with database search. Peak 20 was identified as pantothenic acid, an essential vitamin with *m/z* $[M-H]^-$ at 218.1022 based on database search.

Peak 5 corresponded to hexose because its measured mass with *m/z* at 179.0568 $[M-H]^-$ and fragmentation pattern of 89.0209 and 59.0109 agreed with the reported data (Duangjai *et al*., 2016). Peak 6, 7, and 10 were identified as gluconic acid, quinic acid, and malic acid, respectively, according to their *m/z* $[M-H]^-$ at 195.0521, 191.0561, and 133.0139, respectively (Duangjai *et al*., 2016). Citric acid (14) was detected with *m/z* $[M-H]^-$ at 191.0578 in agreement with a literature data (Duangjai *et al*., 2016)

Peak 27 presented a molecular ion at m/z at 367.1043 $[M-H]^-$, which was identified as 5-O-feruloylquinic acid, a derivative compound of chlorogenic acid commonly found in coffee beans (Jaiswal *et al*., 2010). A cinnamic acid derivative, *p*-coumaric acid (35) was detected with *m/z* 163.0403 $[M-H]^-$ and fragment ion of *m/z* 119.0506 based on comparison with authentic compounds and reported data. It corresponded to the loss of carbon dioxide from precursor ion (Quirantes-Pine *et al*., 2013). Peak 36 was identified as gentisic acid, a dihydroxybenzoic acid and derivative of benzoic acid according to *m/z* $[M-H]^-$ of 153.0194 (Ajila *et al*., 2010). Salicylic acid (42) is a monohydroxybenzoic acid, which is a type of phenolic acid that was detected with *m/z* 137.0250 $[M-H]^-$ in agreement with a previous report by Segarra *et al*. (2006).

Table 1 Mass spectrometry (MS) data of LC-ESI-QTOF-MS spectra and the identification of the GREs monitored in ESI negative mode.

Peak	t_R (min)	Measured mass $[M-H]^-$	Error (ppm)	Molecular formula	Compound identified	Rice varieties/ ethanol concentration (%, v/v)					
						GBR		GRR		GWR	
						70	100	70	100	70	100
1	1.990	158.9787			Unidentified	+	-	+	-	+	-
2	2.392	222.0900			Unidentified	+	+	+	+	+	+
3	2.401	102.0562	-1.43	$C_4H_9NO_2$	γ-Aminobutyric acid (GABA)	+	+	+	+	+	+
4	2.865	131.0454	6.18	$C_4H_8N_2O_3$	D-Asparagine	+	+	+	+	+	+
5	3.145	179.0568	-3.82	$C_6H_{12}O_6$	Hexose	+	+	+	+	+	+
6	3.413	195.0521	4.73	$C_6H_{12}O_7$	Gluconic acid	+	+	+	+	+	+
7	3.647	191.0561	0.06	$C_7H_{12}O_6$	Quinic acid	+	+	+	+	+	+
8	3.943	515.1663	9.38	$C_{30}H_{28}O_8$	Gemichalcone A	+	+	+	+	+	+
9	4.128	194.9480			Unidentified	+	-	+	-	+	-
10	4.374	133.0144	-1.14	$C_4H_6O_5$	Malic acid	+	+	+	+	+	+
11	4.509	130.0889			Unidentified	-	+	-	+	-	+
12	4.607	290.0867	4.95	$C_{11}H_{17}NO_8$	2-Deoxy-2,3-dehydro-N-acetylneuraminic acid	+	+	+	+	+	+
13	4.736	194.9452			Unidentified	+	-	+	-	+	-
14	6.078	191.0204	-3.51	$C_6H_8O_7$	Citric acid	+	+	+	+	+	+
15	6.363	297.1203	-23.71	$C_{18}H_{18}O_4$	2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone	+	+	+	+	+	+
16	6.601	297.1197	-21.19	$C_{18}H_{18}O_4$	7,4'-Dimethoxy-6-C-methylflavanone	+	+	+	+	+	+
17	7.031	164.0725	-4.83	$C_9H_{11}NO_2$	Phenylalanine	+	+	+	+	+	+
18	7.308	147.0660	1.91	$C_6H_{12}O_4$	(R)-2,3-Dihydroxy-3-methylpentanoate	+	+	+	+	+	+

Noted: + Present, - Not present

GBR = germinated black rice; GRR = germinated red rice; GWR = germinated white rice.

Table 1 Mass spectrometry (MS) data of LC-ESI-QTOF-MS spectra and the identification of the GREs monitored in ESI negative mode (Continue).

Peak	t_R (min)	Measured mass $[M-H]^-$	Error (ppm)	Molecular formula	Compound identified	Rice varieties/ ethanol concentration (%, v/v)					
						GBR		GRR		GWR	
						70	100	70	100	70	100
19	7.467	469.1581			Unidentified	+	+	+	+	-	-
20	7.667	218.1040	-2.75	$C_9H_{17}NO_5$	Pantothenic acid	+	+	+	+	+	+
21	7.910	203.0829	-1.46	$C_{11}H_{12}N_2O_2$	Tryptophan	+	+	+	+	+	+
22	8.165	675.2377	-12.21	$C_{33}H_{40}O_{15}$	Acacetin 7-[3'''-(2-methylbutyryl)rutinoside]	+	+	+	+	+	+
23	8.477	395.1589	-4.23	$C_{17}H_{24}N_4O_7$	Tyr Ser Gln	+	+	+	+	+	+
24	8.833	579.1360	-0.79	$C_{26}H_{28}O_{15}$	Quercetin 3-rhamnoside-7-α-L-arabinopyranoside	+	+	+	+	-	-
25	9.076	325.1082	-0.16	$C_{19}H_{18}O_5$	2-Methyl-5,7,8-Trimethoxyisoflavone	+	+	+	+	+	+
26	9.413	563.1433	-4.73	$C_{26}H_{28}O_{14}$	Genistein 7-*O*-glucoside-4'-*O*-apioside	+	+	+	+	+	+
27	9.615	367.1043	-2.29	$C_{17}H_{20}O_9$	5-*O*-Feruloylquinic acid	+	+	+	+	+	+
28	10.003	533.1315	-2.69	$C_{25}H_{26}O_{13}$	Rhamnazin 3-(6''-acetylglucoside)	+	+	+	+	+	+
29	10.367	565.1952			Unidentified	+	+	+	+	+	+
30	10.639	769.2017	-4.11	$C_{37}H_{38}O_{18}$	Isovitexin 2''-*O*-(6'''-feruloyl)glucoside	+	+	+	+	+	+
31	10.806	491.1141	-4.88	$C_{23}H_{24}O_{12}$	Tricin 4'-glucoside	+	+	+	+	+	+
32	11.173	565.1877			Unidentified	+	+	+	+	+	+
33	11.319	477.1064	-5.33	$C_{22}H_{22}O_{12}$	Tricetin 3'-methyl ether 7-glucoside	+	+	+	+	+	+
34	11.492	491.1204	-1.83	$C_{23}H_{24}O_{12}$	Tricin 5-glucoside	+	+	+	+	+	+
35	11.697	163.0403	-1.42	$C_9H_8O_3$	p-Coumaric acid	+	+	+	+	+	+

Note: + Present, - Not present

GBR = germinated black rice; GRR = germinated red rice; GWR = germinated white rice.

Table 1 Mass spectrometry (MS) data of LC-ESI-QTOF-MS spectra and the identification of the GREs monitored in ESI negative mode (Continue).

Peak	t_R (min)	Measured mass $[M-H]^-$	Error (ppm)	Molecular formula	Compound identified	Rice varieties/ ethanol concentration (%, v/v)					
						GBR		GRR		GWR	
						70	100	70	100	70	100
36	12.043	153.0194	-0.44	$C_7H_6O_4$	Gentisic acid	+	+	+	+	+	+
37	12.294	243.1206			Unidentified	+	+	+	+	+	+
38	13.301	187.0966	1.5	$C_9H_{16}O_4$	Methyl N-(a-methylbutyryl)glycine	+	+	-	-	-	-
39	13.301	317.0676	-2.9	$C_{16}H_{14}O_7$	6-Methoxyeriodictyol	+	+	+	+	+	+
40	14.442	540.3328	6.71	$C_{29}H_{51}NO_6S$	(25R)-3α,7α-dihydroxy-5β-cholestan-27-oyl taurine	+	+	+	+	+	+
41	14.843	525.1430	-5.25	$C_{27}H_{26}O_{11}$	Viscutin I	+	+	+	+	+	+
42	15.28	137.0250	-4.22	$C_7H_6O_3$	Salicylic acid	+	+	+	+	+	+
43	16.112	861.2507			Unidentified	+	+	+	+	+	+

Note: + Present, - Not present

GBR = germinated black rice; GRR = germinated red rice; GWR = germinated white rice.

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Peak 8 with *m/z* $[M-H]^-$ at 515.1663 was identified as gemichalcone A (3'- (4-feruloyloxy-3-methylbutyl-2(Z) -enyl) -4,2',4'-trihydroxychalcone), which was a chalcone derivative after the comparison with a Mass Hunter METLIN database search and literature (Liu *et al.,* 2018). Flavonoids are also a major chemical group in GR, thus many derivative forms of flavonoid were detected by LC-ESI-QTOF-MS. The compounds, 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone(15), 7,4'-dimethoxy-6-C-methylflavanone (16), acacetin 7- [3'''- (2-methylbutyryl)rutinoside] (22), 2-methyl-5,7,8-Trimethoxyisoflavone (25), and genistein 7-*O*-glucoside-4'-*O*-apioside (26) were tentatively identified with their $[M-H]^-$ at *m/z* 297.1203, 297.1197, 675.2377, 325.1082, and 563.1433, respectively, by the comparison with literature (Memon *et al.,* 2014) and HMDB database search.

Based on the Mass Hunter METLIN and HMDB database search, peak 12 showed its molecular ion $[M-H]^-$ at *m/z* 290.0867, which was tentatively identified and characterized as 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA). It was reported as an inhibitor of bacterial, viral, and mammalian neuraminidases (REF). Three derivative forms of tricin were detected with *m/z* 491.1141, 477.1064, and 491.1204 $[M-H]^-$, which were identified as tricin 4'-glucoside (31), tricetin 3'-methyl ether 7-glucoside (33), and tricin 5-glucoside (34), respectively, based on the Mass Hunter METLIN and HMDB database search. Peak 39, 40, and 41 corresponded to 6-methoxyeriodictyol, (25R)-3α,7α-dihydroxy-5β-cholestan-27-oyl taurine, and viscutin I, respectively, which presented their molecular ions at *m/z* 317.0676, 540.3328, and 525.1430 $[M-H]^-$, respectively.

All the identified compounds as described above were obtained from all GREs, but there were two compounds were not found in all sample varieties, which were quercetin 3-rhamnoside-7-α-L-arabinopyranoside (24) and methyl *N*-(a-methylbutyry) glycine (38). Quercetin 3-rhamnoside-7-α-L-arabinopyranoside (24) was detected in both extracts from GBR and GRR with its molecular ion $[M-H]^-$ at *m/z* 579.1360. In addition, methyl *N*- (a-methylbutyryl) glycine was detected with its molecular ion $[M-H]^-$ at *m/z* 187.0966 and was presented only in the extracts from GBR sample. Biochemical components in the GR in this study were complementary in agreement with a previous report based on the ^{1}H-NMR analysis (Pramai *et al*., 2018). The tandem mass spectrometry (MS/MS) and NMR analyses were used to elucidate 36 metabolites from rice. Amino acids, organic acids, sugars, phenolics, and flavonoids were the major compounds in GREs from both NMR and LC-MS analysis.

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3.2 GABA content

GABA is an important amino acid in GR and the amount is clearly distinguish compared to those in non-germinated rice (Jannoey *et al*., 2010). In this study, GABA was detected in all GREs corresponded with LC-MS data (Table 1). GABA contents of the GR extracted with 70 and 100 % ethanol concentrations (v/v) are shown in Fig 2A. There was no significant difference of GABA content between the extracts from GBR and GWR for both extraction solvents ($p>0.05$). The lowest GABA content was exhibited in extracts from GRR for both solvents ($p<0.05$). Our preliminary study studied GAD activity in GR samples and the result was in agreement with GABA content. GAD activity of GRR was lower than those of GBR and GWR (data not shown). The result suggested that varieties of rice samples were not affected on GABA content, thus a distinction of GABA content in GR was indicated due to genetic variation of rice varieties (Saikusa *et al*., 1994). A previous study reported that the level of GABA was related to glutamic acid content, because it is synthesized by decarboxylation of glutamic acid catalyzed by GAD enzyme and used as the nitrogen source for the growth of rice and accessed to TCA cycle (Lasse B. *et al*., 2006).

3.3 Total phenolic content (TPC)

The TPC values of GREs using 70 and 100% ethanol concentration (v/v) are shown in Fig 2B. There were significant differences of TPC between the GR extracted with 70% v/v ethanol ($p<0.05$). For the extracts of 70% ethanol (v/v), the highest TPC was presented in GBR, followed by GRR and GWR, respectively ($p<0.05$). A similar pattern of TPC values was observed in samples extracted with 100% ethanol (v/v), where GWR showed the lowest TPC value compared to those of GBR and GRR, which were not significantly different ($p>0.05$). This result was agreement with the LC-MS result that phenolic acid derivatives were detected in all GREs, including 5−O−feruloylquinic acid, *p*−coumaric acid gentisic acid, and salicylic acid. In addition, non-germinated rice both black and red rice varieties were reported as a potent source of phenolic compounds and the amount greater than those in white rice (Pramai and Jiamyangyuen, 2016).

The effect of solvent extraction ratio on TPC values was compared for each rice variety. The results showed that samples extracted with 70% v/v ethanol exhibited higher TPC than those of samples extracted with 100% v/v ethanol ($p<0.05$). The result supported with a previous study by Zhu *et al*. (2011), who reported that 70% v/v ethanol was the most effective solvent to extract phenolic compounds from defatted wheat germ by the comparison with other aqueous ethanol and absolute ethanol. This may be caused by the complex formation of the phenolic compounds with carbohydrates and proteins, which are more soluble in water than other ethanol used for extraction (Zhu *et al*., 2011)

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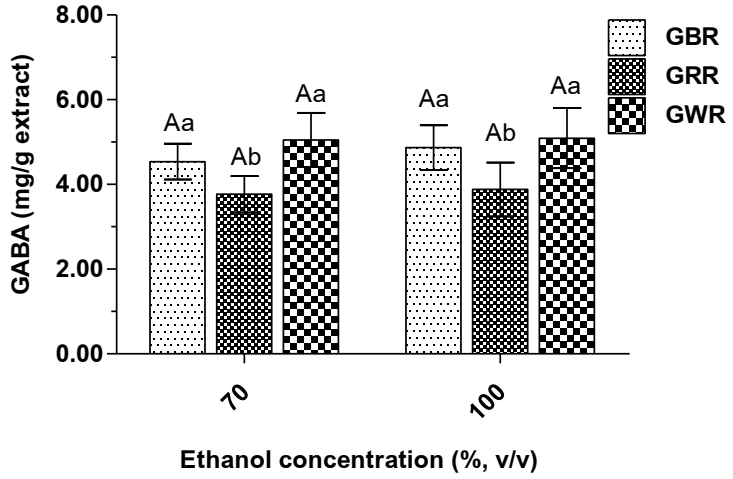
A



B

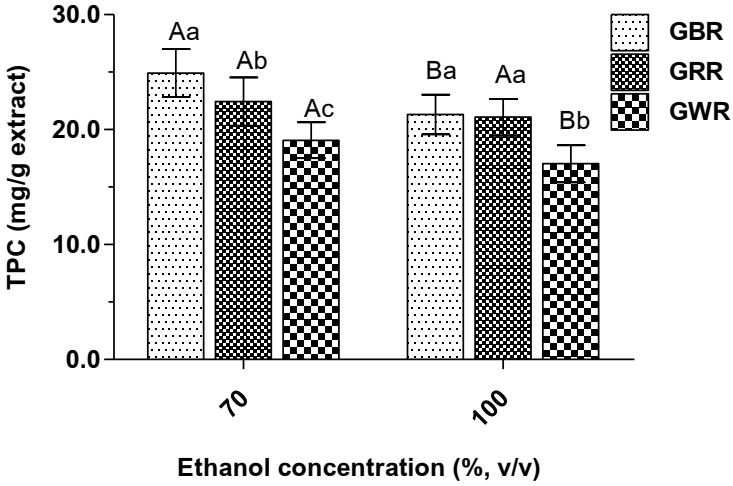


Figure 2 GABA content (A) and total phenolic content (B) of various GR extracted with different concentration of ethanol (n = 6 ± SD). The different capital letters on bars indicate significant differences of ethanol concentrations used for the extraction of the same rice varieties. Different lowercase letters on bars were used to differentiate between rice varieties that were extracted using the same ethanol concentration.

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3.4 Antioxidant properties of germinated rice extracts (GREs)

The antioxidant capacities of GREs using different ethanol concentration are presented in Table 2. Three different antioxidant assays were performed, including DPPH radical scavenging ability, ABTS radical scavenging activity, and ferric reducing antioxidants power (FRAP). Comparing the GR varieties, the extracts from GBR presented the highest DPPH radical scavenging ability, followed by GRR and GWR, respectively (p<0.05). The GBR extracts also contained the greatest ABTS and FRAP values, while these values were not significantly different than the extracts from GRR and GWR (p>0.05). Pramai and Jiamyangyuen (2016) reported that rice grains with black and red pericarp colors contained higher antioxidant activity than the white rice, which agreed with the result in this study. In addition, the antioxidant properties corresponded with LC–MS result that the phenolic acids and flavonoids were characterized in GREs.

Table 2 Antioxidant activities of various GR extracted with two concentrations of ethanol.

GR samples	Ethanol concentrations (%, v/v)	Antioxidant activities (mmol trolox equivalent/g extract)		
		DPPH	ABTS	FRAP
GBR	70	40.00 ± 2.67^{Ab}	50.61 ± 2.56^{Ab}	112.36 ± 4.45^{Ab}
	100	44.95 ± 2.34^{Aa}	58.89 ± 2.62^{Aa}	132.22 ± 5.39^{Aa}
GRR	70	33.72 ± 1.29^{Bb}	45.86 ± 2.49^{Bb}	102.09 ± 4.87^{Ba}
	100	41.97 ± 2.34^{Ba}	48.77 ± 2.10^{Ba}	105.12 ± 4.03^{Ba}
GWR	70	27.82 ± 0.48^{Cb}	44.39 ± 2.47^{Ba}	80.51 ± 4.50^{Cb}
	100	31.79 ± 1.26^{Ca}	47.10 ± 2.07^{Ba}	102.07 ± 6.27^{Ba}

Note: The different capital letters within each column indicate significant differences of rice varieties that were extracted using the same ethanol concentration (n = 6 ± SD).

Different lowercase letters used to differentiate between ethanol concentrations used for the extraction of the same rice varieties (n = 6 ± SD).

DPPH = 2,2–diphenyl–1–picrylhydrazyl radical scavenging ability; ABTS = 2,2'–azinobis (3ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay; FRAP = ferric reducing antioxidant power.

GBR = germinated black rice; GRR = germinated red rice; GWR = germinated white rice.

The DPPH radical scavenging ability of GREs using different ethanol concentrations is presented in Table 2. The GREs using 100% v/v ethanol had higher DPPH value than the extracts using 70% v/v ethanol for all GR varieties (p<0.05). The highest value of the ability to scavenge DPPH radical was observed in GBR variety followed by GRR and GWR varieties, respectively for both ethanol concentrations (p<0.05). The GBR100 demonstrated the greatest

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DPPH value, with an amount of 44.95 mmol trolox /g extract. Absolute ethanol exhibited the powerful solvent for GR in term of DPPH radical scavenging ability. This result was similar to a previous report by Do *et al*. (2014), that studied the antioxidant activities of the extracts of *Limnophila aromatica* using water and various concentrations of methanol, ethanol, and acetone in water. They reported that the extract obtained by 100% v/v ethanol showed the highest DPPH radical scavenging activity. The ABTS radical cation decolourization assay is based on the reduction of ABTS radicals by antioxidant components in plant extracts, which was generally used to assess antioxidant activity. The ABTS radical scavenging activity of GREs was expressed in mmol trolox equivalent/ g extract (Table 2). The extracts from GBR presented the highest ABTS values for both solvent ratios, while ABTS values of extracts from GRR and GWR were not significantly different (p>0.05). The comparison of solvent extraction ratios, 100% v/v ethanol presented better result than 70% v/v ethanol for ABTS values, which corresponded with the result of DPPH scavenging ability as described above. This result was in agreement with a previous study by Zhu *et al*. (2011), that studied ABTS radical scavenging activity of defatted wheat germ extracted by different ratios of ethanol and water. They reported 100% v/v ethanol extract showed the strongest ABTS radical scavenging activity, while the lowest ABTS radical scavenging activity was observed in water extract, since it may contain a smaller amount of active groups than those in ethanol extract.

Ferric reducing antioxidants power (FRAP) assay was based on the reduction of a colorless ferric complex (Fe^{3+} TPTZ) to blue colored ferrous form (Fe^{2+} TPTZ) by the electron donating antioxidants at low pH. FRAP values of GREs are shown in Table 2. In terms of rice varieties, GBR extracts presented the highest FRAP values, while the FRAP values were not significantly different between GRR70, GRR100, and GWR100. A significant difference of FRAP values between samples extracted by 70 and 100% v/v ethanol was found in GBR and GWR samples, but there was no statistical difference between FRAP values from GRR70 and GRR100 (p>0.05). Xu and Chang (2007) studied the effect of extraction solvents on FRAP values in legumes and suggested that 70% v/v ethanol was the best extraction solvent for yellow pea, green pea, and chickpea in term of FRAP assay, which was contradicted to the result in this study. A difference of the result may be attributed to many factors, such as the difference in plant matrix, the polarity index of the used solvents, a number of hydroxyl groups, and the conditions of extraction method that affected on antioxidant capacities in various food systems (Arabshahi-D *et al*., 2007; Pinelo *et al*., 2004; Robards, 2003).

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3.5 Correlation analysis

Pearson's correlation was analyzed to evaluate the relationship between the bioactive components and the different antioxidant assays in GREs (Table 3). A very strong positive Pearson's correlation coefficients were observed between ABTS radical scavenging ability and FRAP (r = 0.935), while DPPH radical scavenging ability presented a strong positive correlation with ABTS radical scavenging ability (r = 0.846) and FRAP (r = 0.883). Phenolic content was moderately correlated with DPPH radical scavenging ability (r = 0.537) and it showed a weak correlation coefficients with the r of 0.302 and 0.404 for ABTS radical scavenging ability and FRAP, respectively. However, it presented the moderate correlation with opposite direction to GABA content (r = −0.533). In addition, the relationship of GABA content with antioxidant activities showed a weak and negligible negative correlations with all antioxidant capacities (Mukaka, 2012).

Table 3 Correlation coefficients (r) between GABA content, total phenolic content, and antioxidant activities in GREs.

Parameters	Correlation coefficient (r)			
	GABA	TPC	DPPH	ABTS
TPC	-0.533			
DPPH	-0.295	0.537		
ABTS	0.162	0.302	0.846*	
FRAP	-0.060	0.404	0.883*	0.935**

Note: ** Significant at 0.01 probability level.

* Significant at 0.05 probability level.

GABA = GABA content; TPC = total phenolic content; DPPH = 2,2−diphenyl−1−picrylhydrazyl radical scavenging ability; ABTS = 2,2'−azinobis (3−ethylbenzothiazoline−6−sulfonic acid) radical scavenging ability; FRAP = ferric reducing antioxidant power.

This study showed a weak to moderate correlations between phenolic constituent and the tested antioxidant activities in GREs, which corresponded with the relationship between the phenolic content and the total antioxidant activity in commonly consumed fruits (Imeh and Khokhar, 2002) and vegetables (Ismail *et al.*, 2004). It was due to the antioxidant capacity observed was not solely from the phenolic contents, but could possibly be due to the presence of some other phytochemicals, such as flavonoids, ascorbic acid, and pigments as well as the synergistic effects among them, which may contribute to the total antioxidant capacity (Sengul *et al.,* 2009). Therefore, the result of this study suggested that the high antioxidant activities in

GREs may be contributed from other compounds besides their phenolic contents, which have not been measured in this work.

4. Conclusions

A powerful analytical technique, LC–ESI–Q–TOF–MS has been applied to identify chemical components of GREs from different solvent extractions. Thirty-three compounds were identified from GR and the major extracted components were sugars, organic acids, amino acids, vitamins, flavonoids, and phenolic acids. These components are nutritional and bioactive products. GABA is an abundant compound that was obtained from all the GR. The strongest antioxidant activities were exhibited in the GR extracted by 100% v/v ethanol. The significant correlation was observed among antioxidant activities and these parameters were low correlated with the phenolic content and GABA. Among the tested rice varieties, GBR samples contained the highest values in term of the content of the antioxidants and activities. Therefore, GR can be promoted as nutraceutical and functional food products.

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References

Abuajah, C.I., Ogbonna, A.C., and Osuji, C.M. 2015. Functional components and medicinal properties of food: a review. Journal of food science and technology. 52(5): 2522-2529.

Ajila, C.M., Jaganmohan Rao, L. and Prasada Rao, U.J.S. 2010. Characterization of bioactive compounds from raw and ripe *Mangifera indica* L. peel extracts. Food and Chemical Toxicology. 48: 3406-3411.

Arabshahi-D, S., Vishalakshi Devi, D. and Urooj, A. 2007. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chemistry. 100(3): 1100-1105.

Benzie, I.F. and Strain, J.J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 299: 15-27.

Ding, J., Ulanov, A.V., Dong, M., Yang, T., Nemzer, B.V., Xiong, S., Zhao, S. and Feng, H. 2018. Enhancement of gama-aminobutyric acid (GABA) and other health-related metabolites in germinated red rice (*Oryza sativa* L.) by ultrasonication. Ultrasonics Sonochemistry. 40: 791-797.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of food and drug analysis. 22(3): 296-302.

Duangjai, A., Suphrom, N., Wungrath, J., Ontawong, A., Nuengchamnong, N. and Yosboonruang, A. 2016. Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (*Coffea arabica* L.) pulp aqueous extracts. Integrative Medicine Research. 5(4): 324-331.

Imeh, U. and Khokhar, S. 2002. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. Journal of Agricultural and Food Chemistry. 50(22): 6301-6306.

Ismail, A., Marjan, Z.M. and Foong, C.W. 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry. 87(4): 581-586.

Jaiswal, R., Patras, M.A., Eravuchira, P.J. and Kuhnert, N. 2010. Profile and characterization of the chlorogenic acids in green Robusta coffee beans by LC-MS^n: identification of seven new classes of compounds. Journal of Agricultural and Food Chemistry. 58: 8722–8737.

Jannoey, P., Niamsup, H., Lumyong, S., Tajima, S., Nomura, M., and Chairote, G. (2010). γ-aminobutyric acid (GABA) accumulations in rice during germination. Chiang Mai Journal of Science. 37(1): 124-133.

Khuhawar, M.Y. and Rajper, A.D. 2003. Liquid chromatographic determination of gamma-aminobutyric acid in cerebrospinal fluid using 2-hydroxynaphthaldehyde as derivatizing reagent. Journal of Chromatography B. 788(2): 413-418.

Kim, H.Y., Hwang, I.G., Kim, T.M., Woo, K.S., Park, D.S., Kim, J.H., Kim, D.J., Lee, J., Lee, Y.R. and Jeong, H.S. 2012. Chemical and functional components in different parts of rough rice (*Oryza sativa* L.) before and after germination. Food Chemistry. 134(1): 288-293.

Kim, J.K., Park, S.Y., Lim, S.H., Yeo, Y., Cho, H.S. and Ha, S.H. 2013. Comparative metabolic profiling of pigmented rice (*Oryza sativa* L.) cultivars reveals primary metabolites are correlated with secondary metabolites. Journal of Cereal Science. 57(1): 14-20.

Lasse K. Bak, Schousboe, A. and Waagepetersen Helle, S. 2006. The glutamate/GABA–glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. Journal of Neurochemistry. 98(3): 641-653.

Liyana-Pathirana, C. and Shahidi, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry. 93(1): 47-56.

Liu, X., Kuang, X.D., He, X.R., Ren, G., Wang, Y., Xu, L.Y., Feng, L.H., Wang, B. and Zhou, Z.W. 2018. Prenylflavonoids from the Twigs of *Artocarpus nigrifolius*. Chemical and Pharmaceutical Bulletin. 66(4): 434-438.

Memon, A.H., Ismail, Z., Aisha, A.F., Al-Suede, F.S.R., Hamil, M.S.R., Hashim, S., Saeed, M.A.A., Laghari, M., Majid, A. and Shah, A.M. 2014. Isolation, characterization, crystal structure elucidation, and anticancer study of dimethyl cardamonin, isolated from *Syzygium campanulatum* Korth. Evidence-Based Complementary and Alternative Medicine, 2014: 1-11.

Moongngarm, A. and Saetung, N. 2010. Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. Food Chemistry. 122(3): 782-788.

Mukaka, M.M. 2012. A guide to appropriate use of correlation coefficient in medical research. Malawi Medical Journal. 24(3): 69-71

Pellati, F., Benvenuti, S., Magro, L., Melegari, M. and Soragni, F. 2004. Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. Journal of Pharmaceutical and Biomedical Analysis. 35(2): 289-301.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M. and Brighenti, F. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. Journal of Nutrition. 133(9): 2812-2819.

Pinelo, M., Manzocco, L., Nuñez, M.J. and Nicoli, M.C. 2004. Interaction among phenols in food fortification: negative synergism on antioxidant capacity. Journal of Agricultural and Food Chemistry. 52(5): 1177-1180.

Piraud, M., Vianey-Saban, C., Petritis, K., Elfakir, C., Steghens, J.P., Morla, A. and Bouchu, D. 2003. ESI-MS/MS analysis of underivatised amino acids: a new tool for the diagnosis of inherited disorders of amino acid metabolism. Fragmentation study of 79 molecules of biological interest in positive and negative ionisation mode. Rapid Communications in Mass Spectrometry. 17: 1297-1311.

Pramai, P., Abdul Hamid, N.A., Mediani, A., Maulidiani, M., Abas, F. and Jiamyangyuen, S. 2018. Metabolite profiling, antioxidant, and α-glucosidase inhibitory activities of germinated rice: nuclear-magnetic-resonance-based metabolomics study. Journal of Food and Drug Analysis. 26(1): 47-57.

Pramai, P. and Jiamyangyuen, S. 2016. Chemometric classification of pigmented rice varieties based on antioxidative properties in relation to color. Songklanakarin Journal of Science and Technology. 38(5): 463-472.

Quirantes-Pine, R., Lozano-Sanchez, J., Herrero, M., Ibanez, E., Segura-Carretero, A. and Fernandez-Gutierrez, A. 2013. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. Phytochemical Analysis. 24(3): 213-223.

Robards, K. 2003. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. Journal of Chromatography A. 1000(1): 657-691.

Saikusa, T., Horino, T. and Mori, Y. 1994. Accumulation of γ-aminobutyric acid (Gaba) in the rice germ during water soaking. Bioscience, Biotechnology, and Biochemistry. 58(12): 2291-2292.

Segarra, G., Jáuregui, O., Casanova, E. and Trillas, I. 2006. Simultaneous quantitative LC–ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. Phytochemistry. 67(4): 395-401.

Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z. and Ercisli, S. 2009. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pakistan Journal of Pharmaceutical Sciences. 22(1): 102-106.

Tian, S., Nakamura, K. and Kayahara, H. 2004. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. Journal of Agricultural and Food Chemistry. 52(15): 4808-4813.

Xu, B.J. and Chang, S.K. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science. 72(2): 159-166.

Yang, Z., Nakabayashi, R., Okazaki, Y., Mori, T., Takamatsu, S., Kitanaka, S., Kikuchi, J. and Saito, K. 2014. Toward better annotation in plant metabolomics: isolation and structure elucidation of 36 specialized metabolites from *Oryza sativa* (rice) by using MS/MS and NMR analyses. Metabolomics. 10(4): 543-555.

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D.A. and Barrow, C.J. 2006. A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. Journal of Applied Phycology. 18(3): 445-450.

Zhu, K.X., Lian, C.X., Guo, X.N., Peng, W. and Zhou, H.M. 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. Food Chemistry. 126(3): 1122-1126