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Original Research Article

Influence of Innovative Processing on γ-Aminobutyric Acid (GABA) Contents, Phytochemicals, and Antioxidant Activity in Flavoured Germinated Rice Powder Products

Rachanee Nammatra[1]* and Chinnaphat Chaloemram[2]

[1] *Production and Quantity Control of Herbal Tea Laboratory, Center of Excellence in Biodiversity Research, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham, 44150, Thailand.*
[2] *Pharmaceutical Chemistry and Natural Product Research Unit, Faculty of Pharmacy, Mahasarakham University, Maha Sarakham 44150, Thailand.*

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ABSTRACT

Germinated rice (GR) is rich in dietary fibre and bioactive compounds, such as GABA and polyphenol compounds, and exhibits antioxidant activity. The objective of this study was to evaluate the levels of GABA, total phenolics, total flavonoids, free phenolics, and free flavonoids, as well as antioxidant activities in germinated rice powder with different flavours. GABA content was determined using LC-MS/MS, antioxidant activities were assessed using DPPH and FRAP assays, and the quantification of free polyphenols was performed using HPLC. The flavoured germinated rice powder products were mixtures of five rice cultivars, combined with four flavours: original, pandan leaves, butterfly pea flowers, and black sesame seed. The results showed that GR with black sesame seed exhibited the highest antioxidant activity (DPPH radical scavenging: 205.21 mg TE/100 g DW; FRAP value: 983.20 mM $FeSO_4$/100 g DW). The highest GABA content was found in the original GR (95.54 ± 1.36 µg/100 g), followed by GR with black sesame seed, GR with pandan, and GR with butterfly pea, respectively. The presence of *p*-coumaric acid, rutin, quercetin, myricetin, and kaempferol was detected in all four flavoured GR samples. Based on the high antioxidant activities and total phenolic and flavonoid content, GR with black sesame seed received the most favourable evaluation. Overall, germinated rice combined with herbal additives, due to its high GABA, phenolic, and flavonoid contents and strong antioxidant activity, holds great potential for the development of functional or health-promoting food products.

Keywords: Germinated rice, *Oryza sativa*, GABA, Polyphenol, Antioxidant

Introduction

Germinated rice (GR), also known as "Khao Hang Ngok" in Thailand, is a rice product processed based on methods used by the Isan Thai people. Germination is a biological process induced by the activation of endogenous enzymes. It begins with a short soak in water, which awakens the dormant grain. During water uptake, the dry seed's metabolic activities are restored, leading to biochemical, nutritional, and sensorial changes. The regulation of various proteins involved in storage reserve degradation, biosynthesis of germination-promoting hormones, detoxification, defence, and cell wall reinforcement promotes seed germination. Differences in water uptake and enzymatic activity exist between plant species.[1, 2] The husk and bran layers of rice grains are rich in bioactive compounds, particularly in pigmented cultivars.[3] Rice (*Oryza sativa*) is a crucial staple food, accounting for 31% of total cereal production worldwide.

*Corresponding author. Email: rachanee.n@msu.ac.th
Tel: +66(619965554)

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A rice grain is composed of bran layers (6-7%), an embryo (2-3%), and an endosperm (approximately 90%).[4] Rice bran and rice germ are high in dietary fibre and bioactive compounds, such as ferulic acid, phenolic compounds, γ-oryzanol, and γ-aminobutyric acid (GABA), and exhibit antioxidant activity.[5] GABA is a free amino acid and a neurotransmitter found in the brain and spinal cord of mammals.[6] In plants, GABA is primarily produced through glutamate decarboxylation by glutamate decarboxylase (GAD). The GAD in rice possesses an autoinhibitory C-terminal domain.[7] The consumption of germinated grains offers numerous advantages. Germinated brown rice, rich in GABA, an anxiety regulator, has a calming effect, may help prevent type 2 diabetes by stimulating insulin production, and exhibits an anti-inflammatory effect in human colon adenocarcinoma cells (Caco-2).[3]

GR also inhibits propyl endopeptidase activity, which may contribute to the prevention of Alzheimer's disease.[8] GR is beneficial for individuals with diabetes while also reducing phytic acid levels and increasing mineral availability. The bioactive compounds in GR are associated with the regulation of blood pressure and heart rate, alleviation of pain and anxiety, improvement of sleeplessness and autonomic disorders associated with menopausal or premenopausal periods, suppression of liver damage, inhibition of cancer cell proliferation, and protection against oxidative stress.[6]

The objective of this study was to evaluate the moisture content, phytochemical content including GABA, phenolic acid compounds, and flavonoid compounds, and antioxidant activity using DPPH and FRAP assays in flavoured germinated rice powder products. The selected analytical methods are appropriate and widely used for accurately assessing bioactive compounds and antioxidant properties, which are crucial indicators of the nutritional quality and functional potential of germinated rice. This study presents a novel approach by integrating germination with flavour enhancement to improve the

phytochemical and antioxidant profiles of rice powder. This combination has been rarely explored in previous studies. The findings may provide new opportunities for tea rice producers and commercial rice growers to promote the development of functional rice products with enhanced health benefits.

Materials and Methods

Materials Collection

Germinated rice (GR) powder, consisting of an equal mixture of five *Oryza sativa* cultivars (Khao Dawk Mali 105, Red Brown Rice, Black Rice, Rice Berry, and Black Sticky Rice), was obtained with four different flavours: GR-1 (original), GR+PD (GR with pandan leaves (*Pandanus amaryllifolius*)), GR+BP (GR with butterfly pea flowers (*Clitoria ternatea*)), and GR+BS (GR with black sesame seed (*Sesamum indicum*)). All samples were sourced locally from At Samat District, Roi Et Province, Thailand (15°54'49.6"N, 103°45'15.0"E).

Chemicals and Reagents

Ethanol-AR grade was obtained from the ITALMAR company (Thailand). HPLC grade acetonitrile was obtained from RCI-Labscan (Thailand). The Folin-Ciocalteu phenol reagent was obtained from Loba (India). A standard γ-aminobutyric acid (GABA, HPLC, ⩾ 97%), trolox, gallic acid, syringic acid, *p*-coumaric acid, rutin, quercetin, catechin, myricetin, and kaempferol were purchased from Sigma Aldrich (Saint Louis, MO, USA). DPPH (1,1-diphenyl-2-picrylhydrazyl) and TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from Sigma Aldrich (Saint Louis, MO, USA).

Method of Extraction

A 100 g sample was weighed and mixed with 1,000 mL of 70% ethanol. The mixture was shaken at 150 rpm at 37 °C for 1 hour, then filtered through Whatman No. 1 filter paper. The extraction was repeated once, and the combined extracts were used for further testing.

Determination of Moisture Content

Moisture content analysis was performed as described by Nielsen.[9] An accurately weighed dried pan with its lid (identifier number noted) was used. Approximately 1 g of the sample was placed in the pan and accurately weighed. The pan was then placed in a forced draft oven at 100 °C for 1 hour. After drying, the pan was stored in a desiccator until the sample reached a constant weight. The percentage moisture (wt/wt) was calculated using the following formula (Equation 1):

$$\%\,\boldsymbol{moisture} = \frac{(\text{wt of wet sample + pan}) - (\text{wt of dried sample + pan})}{(\text{wt of wet sample + pan}) - (\text{wt of pan})} x100 \quad (1)$$

Determination of γ-Aminobutyric acid (GABA)

The method for determining GABA content was modified from El-Naggar et al.[10] and utilised liquid chromatography-mass spectrometry (LC-MS/MS) as described by Eckstein et al.[11] The compounds of interest were detected using a Triple Quadrupole LC-MS/MS LCMS-8030 system (Shimadzu, Kyoto, Japan) operated in positive ion mode with an electrospray ionisation (ESI) source. Mass spectrometry (MS) experiments were performed to isolate and fragment the targeted ions.[12] The optimised operating conditions for the MS detector with a GABA solution were as follows: Interface voltage: 4.5 kV, Drying gas flow: 15 L/min, Nebulizer flow: 3 L/min, Drying gas temperature: 400 °C. The specific ion transition monitored for GABA was m/z 148.0 to m/z 130.0. All data were acquired using LabSolutions software version 5.53 (SP3 Shimadzu). The GABA content was determined by comparing the area under the curve with a GABA standard and was reported as µg/100 g of dried weight.

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method as described by Shen et al.[13] Briefly, aliquots (1.0 mL) of appropriately diluted extracts or standard solutions were mixed with 0.5 mL of 0.5 N Folin-Ciocalteau reagent. The reaction was then neutralised with saturated sodium carbonate solution (75 g/L) and incubated at 25 °C for 2 hours. The absorbance of the resulting blue colour was measured using a spectrophotometer (Shimadzu UV-1780) at 765 nm. A calibration curve was prepared using a gallic acid standard solution. Total phenolic contents were expressed as milligrams of gallic acid equivalents per 100 g of dried weight (mg GAE/100 g DW).

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined using a colorimetric method described by Shen et al.[13] Briefly, 0.5 mL aliquots of appropriately diluted extracts or standard solutions were pipetted into 15 mL polypropylene conical containers containing 2 mL of double-distilled H_2O and mixed with 0.15 mL of 5% $NaNO_2$ solution. After 5 minutes, 0.15 mL of a 10% $AlCl_3 \bullet 6H_2O$ solution was added, and the mixture was allowed to stand for an additional 5 minutes. Then, 1 mL of 1 M NaOH solution was added. The reaction solution was thoroughly mixed and kept for 15 minutes, after which the absorbance at 510 nm was measured. Using a standard rutin calibration curve, the total flavonoid content was calculated and expressed as milligrams of rutin equivalents per 100 g of dried weight (mg RE/100 g DW).

Determination of Free Polyphenols by HPLC

High-performance liquid chromatography (HPLC) was used to perform the quantification of free phenolic acids and flavonoids.[14] The chromatographic system conditions were as follows: mobile phase consisting of 1% acetic acid in water (A) and acetonitrile (B); detector, a photodiode array detector set at 280 and 320 nm; column, a C18 column (4.6 × 150 mm, 5 µm); a flow rate of 1.0 mL/min; and aliquots of 20 µL were injected. The HPLC–DAD system (Shimadzu, Japan) comprised Shimadzu LC-20AC pumps and an SPD-M20A diode array detector. Gradient elution was performed as follows: 0-10 min, 10% B; 10-15 min, 50% B; 15-20 min, 100% B; and a final 5-minute wash. Calibration curves were prepared using phenolic acid standards (gallic acid, syringic acid, and p-coumaric acid) and flavonoid standards (rutin, quercetin, catechin, myricetin, and kaempferol). The concentrations of phenolic acids and flavonoids in the samples were calculated using the respective standard curves.

Antioxidant Activities

DPPH radical scavenging assay: The free radical scavenging activity of the extracts (both free and bound) was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. [14] A 1 mM DPPH solution (180 µL) was mixed with 20 µL of the sample solutions (100 mg/mL) in a 96-well flat-bottomed plate and incubated at room temperature for 15 minutes in the dark. The absorbance of the samples was measured at 517 nm using a microplate reader after incubation. The results were expressed as milligrams of trolox equivalents per 100 g of dry weight (mg TE/100 g DW).

FRAP assay: The ferric reducing antioxidant power (FRAP) assay is based on the reducing ability of antioxidants. A potential oxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}), which then forms a blue complex with TPTZ (Fe^{2+}/TPTZ).[15] Briefly, the FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), a 10 mM solution of TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM hydrochloric acid, and 20 mM iron(III) chloride ($FeCl_3$) in a volume ratio of 10:1:1. In each well of a 96-well flat-bottomed plate, the reagent (180 µL) was mixed with the sample solutions (20 µL, 100 mg/mL) and thoroughly mixed. After 15 minutes, the absorbance was measured at 593 nm. Ferrous sulphate ($FeSO_4$) was used as a standard to establish a calibration curve. The results were expressed as millimoles of $FeSO_4$ equivalents per 100 g of dry weight (mM $FeSO_4$ E/100 g DW).

Statistical Analysis

All analyses were performed in triplicate. Values for different parameters were expressed as the mean ± standard deviation (SD). The data were statistically analysed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The level of significance was set at $p < 0.05$. The prerequisites for conducting this analysis were that the data must be normally distributed, as confirmed by Shapiro-Wilk analysis, and homogeneous, as demonstrated by Levene's test. Statistical analysis was performed using SPSS statistical software. The charts were plotted using GraphPad Prism version 10.1.0. The

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Bioinformatics website (http://www.bioinformatics.com.cn/) was used to visualise the clustering heat map analysis.

Results and Discussion

Moisture Content

In this study, the moisture content percentages of the four flavoured germinated rice (GR) products were analysed (Figure 1). The percentage of moisture content in the original germinated rice (GR-1) was 6.67 ± 2.52%. In germinated rice with pandan (GR+PD) it was 7.67 ± 3.21%, while in germinated rice with butterfly pea (GR+BP) it was 6.00 ± 2.65%, and in germinated rice with black sesame seed (GR+BS) it was 3.67 ± 0.02%. No significant differences in moisture content were observed among the four groups. Notably, all four types of rice exhibited low moisture content, falling within the standard criteria for Thai rice products, which stipulate that the moisture content of all rice types and kinds must not exceed 14 percent.[16] In contrast, Chungcharoen et al. reported that drying at 120°C for 27-33 minutes could reduce the moisture content of germinated rice from 30% to 18% (wet basis).[17]

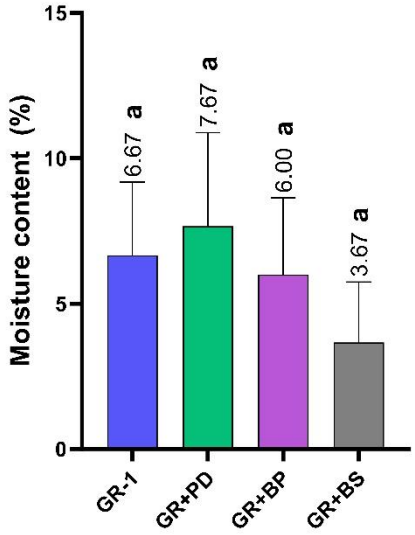

Figure 1: Moisture content in four flavoured GR. Values are expressed as mean ± SD (n=3). Bars with the same letters indicate no significant differences ($p<0.05$).

GABA Content

The GR-1 had the highest γ-aminobutyric acid (GABA) content at 95.54 ± 1.36 µg/100 g DW, followed by GR+BS at 83.02 ± 0.25 µg/100 g DW and GR+PD at 65.17 ± 0.91 µg/100 g DW, respectively. The lowest GABA content was observed in GR+BP at 46.68 ± 0.25 µg/100 g DW (Figure 2). The results showed significant differences in GABA content among the different flavoured GR products. This suggests that the addition of herbs to germinated rice reduced the GABA content compared to the original, unflavoured germinated rice. Consistent with these findings, previous studies have shown that water spraying-based germinated brown rice (Sprayed-GBR) production (10.63 mg/100 g DW) and water soaking-based GBR production (Soaked-GBR) (12.62 mg/100 g DW) resulted in significantly higher GABA content than in brown rice (1.49 mg/100 g DW).[17] Wu et al. reported that the GABA content in germinated paddy rice (GPR) was lower than in germinated brown rice (GBR) at the same germination time (18 to 72 hours), with GBR ranging from 9.35 to 19.21 mg/100 g (DW) and GPR ranging from 9.14 to 14.03 mg/100 g (DW).[18] Moreover, a significant increase in GABA content during rice germination has been reported compared to normal rice seed.[19] However, Tiansawang et al. reported a GABA content of 0.0907 g/kg dry matter in black sesame seed. In this study, the addition of black sesame seed did not increase the GABA content in the GR product.[20]

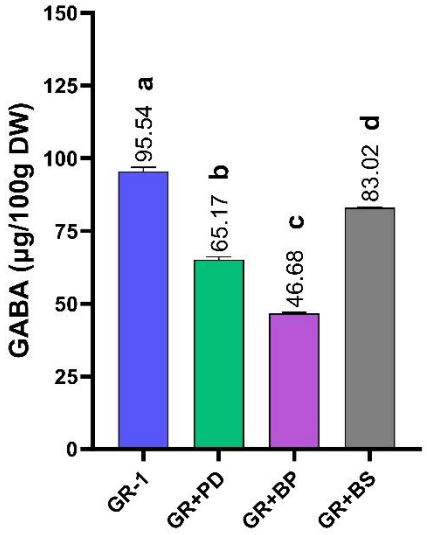

Figure 2: Quantities of GABA in four flavoured GR. Values are expressed as mean ± SD (n=3). Bars with different letters indicate significant differences ($p<0.05$).

Total Phenolic and Flavonoid Content, and Antioxidant Activity

The total phenolic content (TPC) measured by the Folin-Ciocalteau method ranged from 90.12 to 216.26 mg GAE/100 g DW (Figure 3A). The highest phenolic content was found in GR+BS (216.26 ± 3.49 mg GAE/100 g DW), while the lowest was in the GR-1 (90.12 ± 3.55 mg GAE/100 g DW). GR+PD had a phenolic content of 100.52 ± 0.04 mg GAE/100 g DW, and GR+BP had a phenolic content of 103.96 ± 3.59 mg GAE/100 g DW, with no significant differences observed between these two.

The total flavonoid content (TFC) in the germinated rice ranged from 90.20 to 106.41 mg RE/100 g DW (Figure 3B). GR+PD (100.06 ± 0.01 mg RE/100 g DW) and GR+BS (106.41 ± 4.05 mg RE/100 g DW) exhibited the highest total flavonoid contents, with no significant differences between them. However, the GR-1 (99.02 ± 2.56 mg RE/100 g DW) did not show a significant difference compared to GR+PD. Meanwhile, GR+BP (90.20 ± 2.96 mg RE/100 g DW) had the lowest total flavonoid content.

The DPPH radical scavenging activity ranged from 100.46 to 205.21 mg TE/100 g DW (Figure 4A). The highest DPPH inhibition was observed in GR+BS (205.21 ± 6.21 mg TE/100 g DW), followed by GR+BS (129.66 ± 4.46 mg TE/100 g DW), GR-1 (108.99 ± 2.35 mg TE/100 g DW), and GR+PD (100.46 ± 0.08 mg TE/100 g DW), respectively. No significant difference was found between the GR-1 and GR+PD.

The FRAP analysis results are shown in Figure 4B. The highest FRAP value was found in GR+BS (983.20 ± 0.40 mM $FeSO_4$/100 g DW), followed by GR-1 (748.00 ± 11.89 mM $FeSO_4$/100 g DW), GR+BP (727.06 ± 13.05 mM $FeSO_4$/100 g DW), and GR+PD (700.00 ± 0.35 mM $FeSO_4$/100 g DW), respectively.

These results indicate that the addition of herbs to germinated rice generally increases the TPC, TFC, and antioxidant activity compared to the original germinated rice. Previous studies have reported that germinated rice, pandan leaves, butterfly pea flowers, and black sesame seed possess high phenolic and flavonoid content and exhibit moderate antioxidant activity. Suwannatrai et al. found that the TPC and TFC concentrations of roasted broken brown rice powder were 20.40 ± 0.37 mg GAE/g crude extract and 4.58 ± 0.19 mg QE/g crude extract, respectively, and the sample showed high antioxidant activity comparable to a standard Trolox solution.[21]

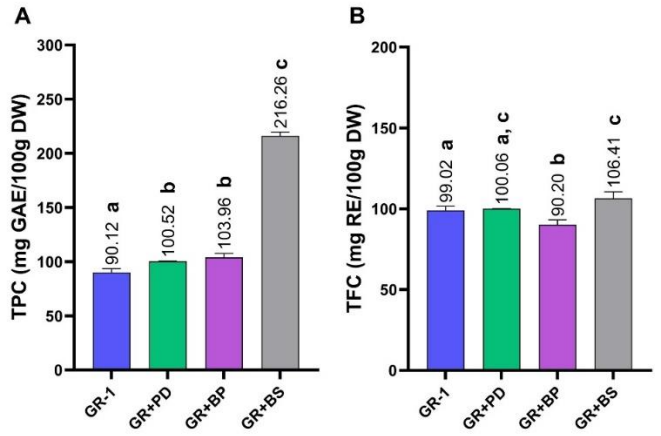

Figure 3: Total phenolic acid (A) and total flavonoid content (B) in four flavoured GR. Values are expressed as mean ± SD (n=3). Bars with the same letters indicate no significant differences ($p<0.05$).

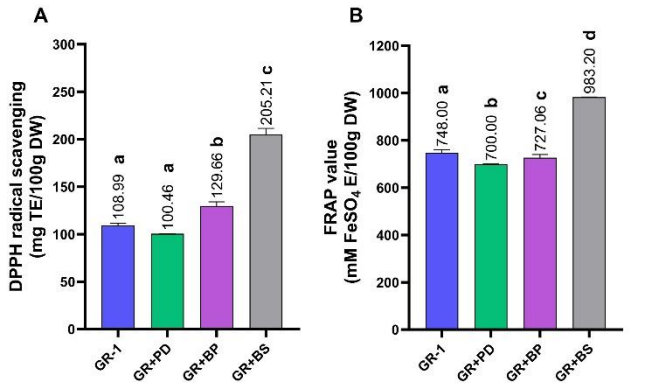

Figure 4: DPPH radical scavenging (A) and FRAP value (B) in four flavoured GR. Values are expressed as mean ± SD (n=3). Bars with the same letters indicate no significant differences ($p<0.05$).

Kammapana[22] studied the phytochemical content and antioxidant activity of ten organic-pigmented rice varieties grown in Surin Province, Thailand, and found significant differences in total phenolic compounds (42.94 to 341.19 mg GAE/100 g DW) and antioxidant activity (2.71 to 34.77 mM TE/100 g DW). Tyagi et al. investigated the antioxidant activity of different coloured rice varieties and found that black rice extract had the lowest IC_{50} values for DPPH and FRAP activity compared to brown and white rice, indicating higher antioxidant activity.[23] Nurhidajah et al. reported that Indonesian black rice contained 25.35 mg QE/100 g flavonoids, 295.56 mg GAE/100 g total phenolic content, and exhibited 61.69% antioxidant activity inhibition.[24]

In our study, the TPCs and TFCs in germinated rice with pandan, germinated rice with butterfly pea, and germinated rice with black sesame seed were higher than in the original germinated rice, with germinated rice with black sesame seed showing the highest TPC and TFC. While black sesame seed extracts have been reported to possess strong antioxidant properties (IC_{50} DPPH: 8.88-44.21 µg/mL; IC_{50} ABTS: 24.91-141.19 µg/mL; EC_{50} FRAP: 222.40 to 872.57 µg/mL), Ruslan et al. found the ethanolic extract of black sesame seed to contain TPC and TFC of 1.57 g GAE/100 g and 0.29 g QE/100 g, respectively.[25] Flower infusion tea composed of butterfly pea (*Clitoria ternatea*) exhibited a TPC of 33.16 ± 0.54 mg GAE/g, a DPPH radical scavenging activity of 39.47 ± 1.78%, and an IC_{50} of 14.41 ± 0.95 mg/mL.[26] The ethanolic extract of pandan (*Pandanus amaryllifolius*) leaf showed TPC and TFC of 38.12 ± 1.49 mg GAE/g and 11.79 ± 0.44 mg QE/g, respectively, with IC_{50} DPPH and ABTS values of 129.32 µg/mL and 104.31 µg/mL.[27]

Quantification of Free Polyphenols

The quantification of free polyphenols was performed using HPLC. The phenolic compound *p*-coumaric acid and three flavonoid compounds (quercetin, myricetin, and kaempferol) were detected in all the GR samples. However, rutin was detected only in the GR+PD and GR+BP groups (Table 1). The *p*-coumaric acid content ranged from 12.65 to 16.04 µg/g, with the highest content found in GR+PD (16.04 ± 0.22 µg/g), followed by GR+BP (14.76 ± 0.50 µg/g), GR+BS (13.53 ± 0.16 µg/g), and GR-1 (12.65 ± 0.55 µg/g), respectively. No significant difference in *p*-coumaric acid content was observed between GR+BS and GR-1.

GR+BP exhibited the highest rutin content (196.42 ± 3.42 µg/g), followed by GR+PD (52.79 ± 1.26 µg/g). Quercetin content ranged from 29.21 to 109.43 µg/g, with the highest content found in GR+PD (109.43 ± 0.66 µg/g), followed by GR+BS (100.67 ± 0.42 µg/g), and GR-1 (57.31 ± 2.03 µg/g). The lowest quercetin content was found in GR+BP (29.21 ± 0.93 µg/g). Myricetin content ranged from 5.18 to 29.64 µg/g, with the highest content found in GR+BP (29.64 ± 0.32 µg/g), followed by GR+PD (8.04 ± 0.25 µg/g), and GR+BS (7.55 ± 0.03 µg/g). The lowest myricetin content was found in GR-1 (5.18 ± 0.30 µg/g). No significant difference in myricetin content was observed between GR+PD and GR+BS. Kaempferol content ranged from 5.78 to 109.20 µg/g, with the highest content found in GR+BP (109.20 ± 0.51 µg/g), followed by GR-1 (13.30 ± 0.37 µg/g), and GR+BS (9.70 ± 0.24 µg/g). The lowest kaempferol content was found in GR+PD (5.78 ± 0.31 µg/g). Consistent with previous studies, Butsat and Siriamornpun found that rice bran, rice husk, brown rice, and milled rice of Khao Dawk Mali 105 contained ferulic acid, vanillic acid, and *p*-coumaric acid as three major phenolic acids.[28] Meanwhile, Punnongwa et al. reported the detection of phenolic compounds including gallic acid, trans-ferulic acid, protocatechuic acid, vanillic acid, coumaric acid, hydroxy-benzoic acid, syringic acid, and sinapic acid, as well as the flavonoid compound quercetin, in germinated black rice.[29] Pramai et al. also reported the detection of phenolic acids including *p*-coumaric acid, gentisic acid, and salicylic acid in germinated white rice, germinated black rice, and germinated red rice.[30] Additionally, Yamuangmorn et al. found phenolic compounds including protocatechuic acid, caffeic acid, syringic acid, and ferulic acid, and flavonoid compounds including rutin, quercetin, kaempferol, apigenin, and luteolin in fresh and year-old seeds during rice germination.[31] Moreover, butterfly-pea flower (*Clitoria ternatea*) is a natural source of phenolic compounds, flavonoids, and anthocyanins.[32,33] Sesame seed (*Sesamum indicum*) is a natural source of lignans and some free phenolic acids such as chlorogenic, ellagic, gallic, caffeic, ferulic, and *p*-coumaric acids, as well as flavonoids (i.e., rutin, quercetin, and apigenin).[34] Meanwhile, pandan leaves (*Pandanus amaryllifolius*) are a natural source of fragrant compounds such as pandanamine, coumarin, and ethyl vanillin,[35] and phenolic and flavonoid compounds.[36] It is plausible that adding other herbs to germinated rice increases the levels of certain free phenolic acid and flavonoid compounds compared to not adding herbs, depending on the specific compounds present in the added herbs. Polyphenol compounds from plants, including phenolics and flavonoids, possess antioxidant properties that help reduce risk factors associated with various diseases. Furthermore, these compounds are classified as secondary metabolites, derived from phenylalanine and tyrosine, and are widely distributed in plants. They exhibit diverse properties and are believed to have beneficial effects on human health.[37,38]

The hierarchical clustering heat map (Figure 5) of the four flavoured GR products effectively distinguished patterns in phytochemicals and antioxidant activity, revealing three distinct clusters. Cluster 1 highlighted the GR-1 and GR+PD, characterised by high moisture content, but with high GABA content in the GR-1 and high quercetin content in GR+PD. Cluster 2 highlighted the GR+BP, demonstrating high rutin, myricetin, and kaempferol content.

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Table 1: Quantities of free polyphenol in four flavoured GR

Free polyphenols (µg/g DW)	Germinated rice powder products			
	GR-1	GR + PD	GR + BP	GR + BS
Phenolic acid compounds				
Gallic acid	ND	ND	ND	ND
Syringic acid	ND	ND	ND	ND
p-coumaric acid	12.65 ± 0.55^{a}	16.04 ± 0.22^{b}	14.76 ± 0.50^{c}	13.53 ± 0.16^{a}
Flavonoid compounds				
Rutin	ND	52.79 ± 1.26^{a}	196.42 ± 3.46^{b}	ND
Quercetin	57.31 ± 2.03^{a}	109.43 ± 0.66^{b}	29.21 ± 0.93^{c}	100.67 ± 0.42^{d}
Catechin	ND	ND	ND	ND
Myricetin	5.18 ± 0.30^{a}	8.04 ± 0.25^{b}	29.64 ± 0.32^{c}	7.55 ± 0.03^{b}
Kaempferol	13.30 ± 0.37^{a}	5.78 ± 0.31^{b}	109.20 ± 0.51^{c}	9.70 ± 0.24^{d}

Abbreviation: GR-1: germinated rice; GR + PD: Germinated rice with pandan; GR + BP: Germinated rice with butterfly pea; GR + BS: Germinated rice with black sesame seed; ND: not detected. Values are expressed as mean ± SD of triplicate measurements (n = 3). Different superscript letters (^{a}, ^{b}, ^{c}, ^{d}) in the row represent significant differences (p<0.05).

Cluster 3 highlighted the GR+BS, demonstrating high total phenolic content, total flavonoid content, DPPH radical scavenging activity, FRAP value, quercetin, and GABA content, along with low moisture content.

This study suggests that adding herbs to germinated rice did not significantly alter the moisture content but reduced the GABA content compared to the original germinated rice. However, the addition of herbs to germinated rice generally increased the total phenolic content, total flavonoid content, DPPH inhibition, FRAP value, and the levels of specific free phenolic acid and flavonoid compounds compared to the original germinated rice. The preceding discussion indicates that adding herbs to germinated rice resulted in a reduction of GABA but an increase in polyphenol content, which contributes to bioactive compounds with antioxidant activity. Thus, this presents a selective choice for further *in vivo* studies on antioxidant and other pharmacological activities.

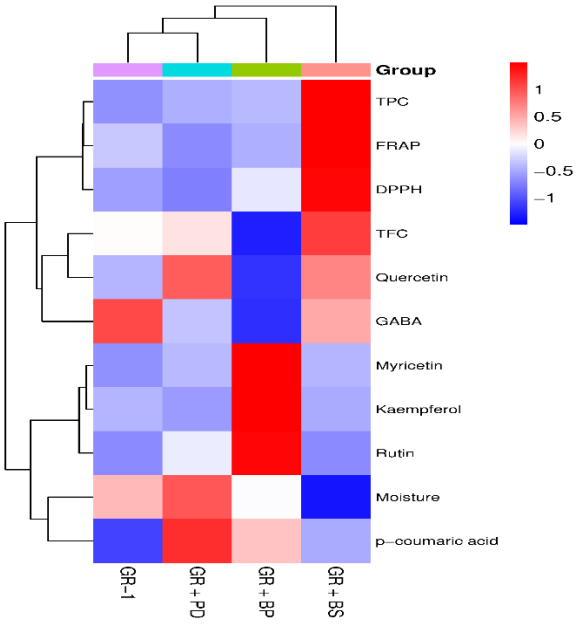

Figure 5: Heat map cluster analysis for exploring the columns of four flavoured GR in relation to their phytochemicals and antioxidant activity.

Conclusion

The addition of herbs to germinated rice did not increase the GABA content; however, it did enhance the polyphenol content, which comprises bioactive compounds with antioxidant activity. Among the flavoured germinated rice products, the black sesame seed variant received the most favourable evaluation based on its high phytochemical and antioxidant properties. This research provides fundamental information for the assessment of functional foods derived from germinated rice. Given its substantial GABA, phenolic, and flavonoid content, as well as its antioxidant activity, germinated rice holds significant potential for the development of healthy food products. It also enhances the value of local rice products, promoting their development into sustainable commercial goods.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any responsibility for claims relating to the content of this article shall be borne by them.

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