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Values, bioactive compound, antioxidant activities of brown rice and germinated brown rice, and the pellet products

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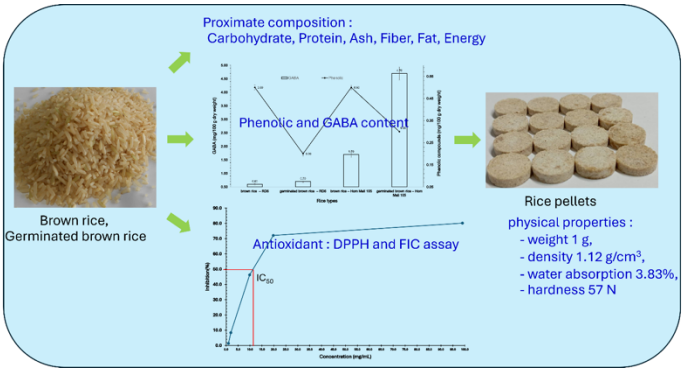
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Abstract

This study aimed to explore the dietary components, phenolic compounds, GABA content, and antioxidant capacity of brown rice and germinated brown rice from two rice varieties: the glutinous rice variety Kor Khor 6 (RD6) and the paddy rice type Hom Mali 105 rice. The study findings indicated that protein composition varied between 6.76 – 7.91%, fat content ranged from 2.49 – 2.85%, and carbohydrate content varied from 73.65 – 79.83%. The average energy value is 362 kcal 100 g $^{-1}$. Germinated brown rice of Hom Mali 105 has the highest GABA levels with 4.70 mg 100 g^{-1} dry weight. The Hom Mali 105 exhibited the highest levels of phenolic compounds, registering at 0.5 mg 100 g^{-1} dry weight. Germinated brown rice of Hom Mali 105 has the highest capacity to bind to DPPH free radicals and Fe^{2+}, which IC50 values of 7.12 and 82.00 mg mL^{-1}, respectively. Following the research on rice pelleting, the result of pellets was discovered to be completely round and undamaged. This study shows that brown rice and germinated brown rice are good sources of nutrients and energy. They also possess potent antioxidant qualities. Preliminary rice powder pellets were produced from germinated brown rice of Hom Mali 105. These rice pellets can be molded into uniquely shaped products. The solid density, water absorption, and hardness of the rice pellets were measured at 1.12 ± 0.05 g cm^{-3}, 3.83 ± 1.02 %, and 57.86 ± 5.88 N, respectively. These pellets are easy to consume and offer a complete range of nutrients.



Keyword: Phenolic; GABA; Germinated brown rice; Antioxidant; Proximate; Rice pellets

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1. Introduction

The primary energy source for most people in Southeast Asia is rice [1]. It is grown in many countries, including Thailand. There's certainly a lot of rice cultivation in the different provinces in Thailand. The northeastern region in Sakon Nakhon and Nakhon Phanom provinces are good sources of quality rice cultivation because of their location. These areas are surrounded by mountains and rivers passing through that makes the soil quality appropriate for farming. Rice that is highly popular in the Northeast is sticky rice RD6 and paddy rice Hom Mali 105 because it is smooth and fragrant. The province of Sakon Nakhon produces a wide variety of rice products, including brown rice, germinated brown rice [2], and Khao Mao [3]. However, selling paddy rice is the main source of revenue for most farmers. The price of sold rice is not worth the investment. Farmers are hence still unable to achieve sustainable self-reliance.

Rice is a wonderful source of lipids, carbs, and protein. Along with other necessary physiologically active ingredients including vitamin B, phenolic compounds, good fats, and minerals [1, 4-6], germinated rice also includes gamma aminobutyric acid (GABA) [7-9]. Aside from rice being our staple food, it also contains medicinal benefits due to its special properties, which include lowering cholesterol, crushing free radicals, avoiding heart disease, and preventing cancer [10]. Furthermore, a study revealed that consuming germinated brown rice on a regular basis can help lower blood pressure, prevent headaches, prevent cancer of the large intestine, regulate blood sugar levels, and accelerate the metabolism of the brain and including preventing Alzheimer's disease [11-13]. Rice is more than just food to be eaten after being cooked. It is also processed to make a multitude of products, such as canned rice, rice flour, and noodles. Even though rice is turned into various foods, it is still not a food that is easily or conveniently consumed. It's not deemed ready to eat until it's cooked. These data led the research team towards the conclusion that it is critical to develop a quick and simple substitute food that provides complete nutrition and can be consumed anywhere, at any time, by everyone. That's pellets of rice. This will help solve the problem of young children and the elderly experiencing difficulties with eating. Moreover, this could lead to the development of rice as a food source for astronauts. Since a few studies on the creation of rice pellets were published [14]. The researchers reported that the tablet product made from broken Riceberry rice, which consumers prefer and is suitable for tablet compression, consists of a formula containing 30% rice, 18% milk powder, 30% icing sugar, and either 20% maltodextrin or 20% strawberry powder [14]. Nevertheless, there have been no reports of rice pellets having nutrient-dense and bioactive components.

Therefore, this study looked at the nutrition, phenolic compounds, GABA and antioxidant activity in brown rice and germinated brown rice, and production instructions for rice pellets, which may be another way for farmers to produce rice to raise the price of Hom Mali 105 rice and RD 6 glutinous rice.

2. Materials and Methods

Chemical

The Folin-Ciocalteu phenol reagent, Gamma-aminobutyric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferrozine were acquired from Sigma-Aldrich Chemical Co., USA. The remaining chemicals used were of analytical grade.

Sample preparation

In this study, the rice investigated samples were the glutinous rice variety Kor Khor 6 (RD6) and the Hom Mali 105 rice from the districts of Sakon Nakhon and Nakhon Phanom in Thailand. Rice was soaked in water at a ratio of 1:4 w w^{-1} for 12 h. The soaking water was discarded, rinsed, and was evenly spread over a damp cheesecloth. Then, it was covered with another damp cheesecloth and was left to dry at 30°C for 24 hours. Subsequently, a hot air dryer set to 55°C was used for 10 h to complete the drying process. Finally, the dried rice was

broken open to remove the husks, resulting in germinated brown rice. For the preparation of brown rice, husks can be cracked and removed without the necessity for germination.

Proximate analysis

Protein, fat, ash, fiber, carbs, moisture, and energy were used to make up the proximate analysis. This proximate analysis method is based on a report on nutritional values and their potential applications in food products of Krabok Seed (*Irvingia malayana*) published by Preecharram et al. [15] and AOAC 2000 method [16].

Total phenolic compounds analysis

The method followed to analyze total phenolic compounds was described in the 2023, published by Preecharram et al. [3].

Gamma Aminobutyric acid analysis

The GABA contents were prepared following the protocols outlined by Jandaruang et al.,[2]. In brief, each crude extract was combined with 20 µL of 0.2 M borate buffer pH 9.4 and 100 µL of 6% phenol. The resulting solutions were thoroughly mixed and then cooled in a cooling bath for 5 min. Subsequently, 30 µL of 10% CaOCl was added, and the solution was vigorously shaken for 1 min before being cooled again in a cooling bath for 5 min. To complete the process, the solution was boiled at 90°C for 1 min and then allowed to cool. The optical density was measured at a wavelength of 630 nm, using methanol as a blank. The quantification of GABA content was performed by comparing the optical density readings with the standard GABA content curve.

Antioxidant assay

Antioxidant activity was analyzed using two methods: DPPH assay and ferrous ion chelating (FIC). The DPPH method was performed as reported by Preecharram et al., [3]. The FIC assay was conducted following the modified method of Nurliyana et al., [17]. In briefly, 250 µL aliquots of each crude extract were combined with 25 µL of 2 mM $FeCl_2$ and 800 µL of 70% ethanol. The mixtures were thoroughly blended and incubated for 5 min. Subsequently, 100 µL of 5 mM ferrozine was added, mixed, and left in the dark at room temperature for an additional 5 min. The absorbance of the Fe^{2+}-ferrozine complex was then measured at 562 nm against 70% ethanol as the blank. For the negative control, the reaction mixture included only $FeCl_2$, 70% ethanol, and ferrozine.

The chelating effect was determined using the following equation [17] : Chelating effect (%) = [(Acontrol–Asample)x100]/Acontrol (1) Acontrol is absorbance of the reaction mixture including only $FeCl_2$, 70% ethanol, and ferrozine. Asample is absorbance of the reaction mixture with crude extract, $FeCl_2$, 70% ethanol, and ferrozine.

Pellet rice preparation

Germinated brown rice, Hom Mali 105, was roasted and ground until it became very smooth. After that, 30 g of rice powder, 20 g of maltodextrin, 40 g of icing sugar, 1 g of magnesium stearate, and 9 g of milk powder are measured out. The various ingredients are combined using a dry blender. The ingredients are put inside the pellet maker. Finally, the finished pellet goods are transferred into aluminum foil bags.

Physical properties of rice pellets

Weight

Each of the ten rice pellets is weighed separately using a 4-position scale to determine their average weight.

Thickness and size

The thickness and radius of ten rice pellets should be measured with a vernier caliper, and the results should be averaged.

Solid density

The density of a solid is calculated from the formula;

$$M / (3.14 \times r^2 \times h) \quad (2)$$

where M is the sample weight (g), h is the sample thickness (cm) and r is the radius (cm)[18].

Water absorption

The weighing of the rice pellets was conducted prior to their initiation of water absorption. After being submerged in water at 27°C for 30 s, ensuring that the water level in the container is at least 25 mm above the sample, the sample is removed from the water. The sample is then blotted with a clean cloth and weighed after water absorption. Following this, the water absorption value (%) is calculated using the formula;

Water absorption(%) =

$$[(w2 - w1) / w1] \times 100 \quad (3)$$

where the weights before and after water absorption are denoted by the numbers w1 and w2, respectively.

Hardness

The average hardness can be obtained by using a hardness tester (Hardness Tester-TBH125, ERWEKA, Germany).

3. Results and Discussion

Proximate content

The primary nutritional composition of germinated brown rice and brown rice is shown in Table 1.

Table 1 Proximate content of brown rice and germinated brown rice.

Samples	Proximate content (%)					
	Ash	fiber	Crude fat	Protein	Moisture	Carbohydrate
Brown rice – RD6	1.32± 0.09	1.30± 0.11	2.85± 0.29	7.16± 0.10	13.73± 0.14	73.65± 0.75
Germinated brown rice – RD6	1.30± 0.06	1.32± 0.08	2.75± 0.18	6.76± 0.17	11.20± 0.11	76.67± 0.66
Brown rice – Hom Mali 105	1.29± 0.06	1.20± 0.21	2.67± 0.14	7.24± 0.22	7.75± 0.20	79.83± 0.89
Germinated brown rice – Hom Mali 105	1.09± 0.07	1.44± 0.17	2.49± 0.12	7.91± 0.28	7.80± 0.18	79.28± 0.43

Values are presented as Mean ± SD

The ash percentage of brown rice and germinated brown rice ranges from 1.09% to 1.32%, as Table 1 illustrates. These values were lower than those of Pakistani rice cultivars (*Oryza sativa* L.), which showed a range of 1.48% to 1.98% [19] and lower than Nepal brown rice of 1.7% [20]. Ash content is a measurement of the quantity of inorganic stuff that is left over after all organic matter has completely broken down at high temperatures. It is useful for estimating the amount of minerals in food. Minerals are nutrients that are deficient in energy. It is a nutrient that is essential to the body's ability to strengthen and regulate the operation of many different parts, including hormone regulation and the regulation of muscle function [21]. The amount of ash in rice is a good indicator of the mineral elements, including sodium, magnesium, calcium, and potassium [22].

The crude fiber content of brown rice and germinated brown rice ranged from 1.20% to 1.44% (Table 1). These fiber content values were greater than those of Pakistani rice cultivars (*Oryza sativa* L.), which showed a range of 0.71% to 0.92% [19]. Rice fiber is composed of pectin (soluble), lignin, hemicelluloses, and cellulose (insoluble) [1]. Insoluble dietary fiber can absorb significant amounts of water and induce peristalsis in the large intestine, which speeds up the body's removal of various garbage and toxins. Furthermore, eating foods that contain dietary fiber will lower blood cholesterol levels, which reduces the risk of heart disease and stroke [11].

The soxhlet extraction analysis revealed that brown rice and germinated brown rice contained 2.49% to 2.85% fat (Table 1) which is close approximately to the 3% fat content of plain rice [1]. The fats which are found are unsaturated fats like oleic acid, linoleic acid, and eicosatrienoic acid and saturated fats such as myristic acid, palmitic acid, and stearic acid [6]. Rice bran oil has been shown to prevent diabetes and cancer [23, 24].

The Kjeldahl method uses the total nitrogen measurement to determine the protein content of food samples. Table 1 displays data indicating that the crude protein levels of brown rice and germinated brown rice ranged from 6.76% to 7.91%. Our results align with the findings of Sen et al. [4], indicating that rice cultivars exhibit a protein content ranging from 7-8%. Additionally, rice is known to contain

significant levels of prolamine, glutelin, globulin, albumin, soluble protein, and crude protein [1].

The moisture content in both brown rice and germinated brown rice varies between 7.75% and 13.73% (Table 1). According to the general guideline for dry foods, the moisture content should not surpass 15%. This guideline applies to both germinated brown rice and brown rice. The minimal moisture levels contribute to prolonged storage times but also contributes to the reduction of microbial contamination and enzyme activity.

Brown rice and germinated brown rice have estimated carbohydrate contents ranging from 73.65% to 79.83% (Table 1). These carbohydrate content values are comparable to those of the *Oryza sativa* L. rice varieties from Pakistan, which ranged from 76.98% to 79.43% [19]. The primary carbohydrates found in rice are amylose and amylopectin [1].

The energy values were computed based on each species' nutritional content (Table 2). The energy value of brown rice and germinated brown rice is between 348.88 and 372.48 kcal, which is comparable to the 384 kcal of brown rice that Juliano reported [25]. As a primary energy source, rice provides the highest energy density through carbohydrates, followed by protein and fat in the second and third positions, respectively.

Table 2 Energy values of brown rice and germinated brown rice.

Samples	Energy (kcal)			Total energy (kcal)
	Carbohydrate	Protein	Fat	
Brown rice – RD6	294.58± 2.55	28.62± 0.11	2.85± 0.19	348.88± 4.58
Germinated brown rice – RD6	306.66± 3.68	27.05± 0.07	2.75± 0.23	358.44± 5.81
Brown rice – Hom Mali 105	319.33± 5.66	28.96± 0.33	2.69± 0.21	372.48± 4.73
Germinated brown rice – Hom Mali 105	317.13± 4.71	31.62± 0.20	2.49± 0.19	371.14± 4.22

Values are presented as Mean ± SD

Total phenolic compound

The Folin-Ciocalteu technique was used to analyze the total phenolic compounds. The total phenolic compounds in brown rice and germinated brown rice varied between 0.20 to 0.50 mg 100 g^{-1} dry weight (Fig. 1). The total phenolic compounds in brown rice were higher than those in germinated brown rice. The total amount of phenolic compounds in this study was less than that of Khao-Mao, which contained 0.59 to 0.79 mg g^{-1} of dry weight. [3]. Several phenolic compounds, including as ferulic acid, coumaric acid [26], and cinnamic acid are commonly found in rice [27]. Numerous biological activities of cinnamic acid include its antiviral, antifungal, antibacterial, and antioxidant properties [28], as well as its stimulation of insulin secretion, enhancement of pancreatic cell function, inhibition of liver gluconeogenesis, delay in the breakdown of carbohydrates and glucose absorption, and prevention of diabetes and its complications [29].

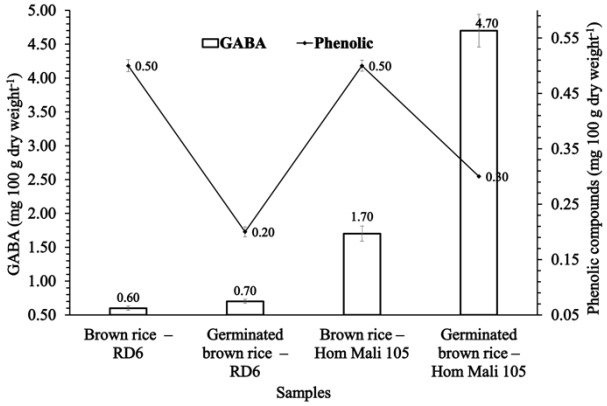


Fig. 1 Total phenolic compound contents (line ——) and GABA contents (bar ▭) of brown rice and germinated brown rice.

Gamma-aminobutyric acid content

The GABA contents of brown rice and germinated brown rice were observed to differ. Figure 1 shows that each sample's GABA level ranges from 0.60 to 4.70 mg 100 g^{-1} dry weight. The maximum GABA concentration found in germinated brown rice-Hom Mali 105, followed by 1.70 mg 100 g^{-1} dry weight of brown rice-Hom Mali 105. The results show that germination boosts the concentration of GABA. This is because GABA is produced during the germination process by the enzyme glutamate decarboxylase, which uses a decarboxylation reaction to transform the L-glutamic acid into GABA. The GABA

content of traditional rice varieties vary widely, from 0.36 to 5.1 mg 100 g^{-1} [30-33]. GABA has several positive effects on health and is a necessary inhibitory neurotransmitter in mammals' central nervous systems [34].

Antioxidant activities

The antioxidant activity of the samples was assessed through two methods: DPPH assay, which directly interacts with free radicals, and FIC, targeting ferrous, a metal known to induce free radical formation. Results indicated that the IC50 value for the DPPH method ranged from 7.12 to 9.30 mg mL^{-1}, whereas the IC50 for the FIC method ranged from 82.00 to 121.00 mg mL^{-1} (Fig. 2). Among the varieties examined, germinated brown rice - Hom Mali 105 displayed the highest antioxidant activity, with brown rice – RD6 following closely in the DPPH method. In terms of iron binding capacity, brown rice – Hom Mali 105 demonstrated the highest capacity, followed by brown rice – RD6.

The superior capacity of germinated brown rice – Hom Mali 105 to bind with the DPPH free radical may be attributed to the influence of GABA. GABA has the capability to donate electrons at the carboxylic position to free radicals. In the case of brown rice – Hom Mali 105, its outstanding ability to bind with ferrous may be a result of the presence of phenolic compounds. As per Guo et al. [35], phenolic compounds exhibit specific functional sites capable of binding transition metals within their structure. Examples include the 4-keto group paired with a 3-and/or 5-hydroxyl group, or the presence of a 7–8 o-dihydroxyphenyl (catechol) structure in the benzene ring of flavonoids, as well as certain types of substituents [36]. Phenolic compounds with the mentioned structure, such as gallic acids, can establish robust bindings with Fe^{2+}. Certainly, the inclusion of methoxyl and hydroxyl groups in the ortho position of phenolic acids enhances their ability to chelate Cu^{2+} [37]. Hence, the capability to chelate Fe^{2+} relies on the phenolic structure, as well as the location and quantity of hydroxyl groups.

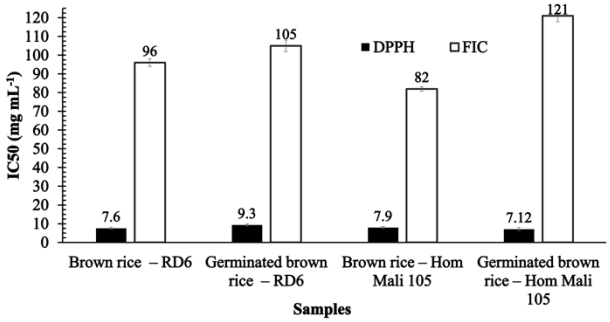


Fig. 2 Antioxidant activities of brown rice and germinated brown rice analyzed by DPPH assay and FIC assay.

Physical properties of rice pellets

The resultant rice pellets are hard, sturdy, and spherical (Fig. 3). By examining the weight, thickness, radius, solid density, water absorption, and hardness value of the rice pellets. The rice pellets exhibited the following parameters: a thickness of 0.44 cm, a radius of 0.84 cm, a solid density of 1.12 g cm^{-3}, an average weight of 1.03 g before water absorption and 1.07 g after water absorption, and a hardness value 57.86 N (Table 3).

The correct ratio, as indicated by Samsalee and Mangklanan [14], is to use 30 g of rice powder from the pelletizing ingredients. Exceeding this amount can lead to easy pellet shattering. Formulas containing more than 50% rice powder are deemed unsuitable for pellet processing, as per the findings of the study [14].

Upon physical examination, the rice pellets displayed an average weight of approximately one gram. While the solid density exceeded that of commercially available A1 milk pellets, it remained lower than that of A2 milk pellets. The water absorption value for the studied rice pellets surpassed those of A1 and A2 milk pellet types (Table 3). This water absorption value is indicative of solubility and bioavailability in the body. The hardness grade of 57.86 N for the rice pellets in the study falls within the suggested range of 29–69 N, which is often preferred by children to eat [38]. The appropriate usage of the product, such as in ingested tablet, lozenge, or chewable tablet forms, is crucial to ensure that the pellets remain intact during production and transit. However, potential future improvements to the pellet recipe could be

explored to enhance its appeal, including the incorporation of natural flavor and coloring ingredients.

Table 3 physical properties of rice pellets.

Sample	Weight (W1) (g)	Weight (W2) (g)	Thickness (cm)	Radius (cm)	solid density (gcm^{-3})	water absorption (%)	Hardness (N)
Rice pellet	1.03± 0.04	1.07± 0.05	0.44± 0.02	0.84± 0.00	1.12± 0.05	3.83± 1.02	57.86± 5.88
A1*	1.33± 0.02	1.36± 0.02	1.81± 0.02	0.59± 0.00	0.67± 0.00	2.17± 0.03	ND
A2*	1.14± 0.01	1.15± 0.01	0.41± 0.00	0.86± 0.00	1.21± 0.11	1.05± 0.02	ND

Values are presented as Mean ± SD,
ND; Not determined
A1* is a type of milk tablet that's widely available, brand1.
A2* is a type of milk tablet that's widely available, brand2.

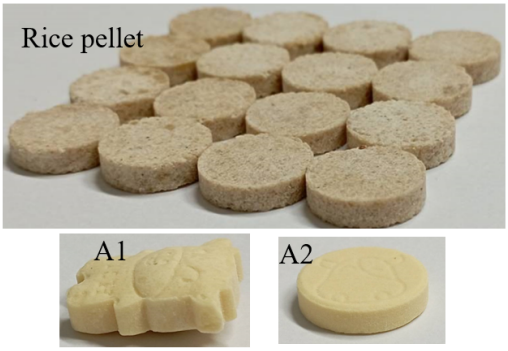


Fig. 3 Characteristics of pellet rice made from roasted germinated brown rice – Hom Mali 105, milk tablet : A1 and A2.

4. Conclusion

The nutritional content of glutinous RD6 strain brown rice and Hom Mali 105 rice is notably high. The energy content ranges from 350 to 370 kcal. Moreover, rice contains GABA and phenolic compounds, both known for their biological activity. GABA has the capability to directly bind free radicals and metals that produce free radicals. In addition to examining the nutritional quality and bioactive components of rice, we explored the convenient ways to consume rice, such as with rice pellets. The currently manufactured rice pellets exhibit ideal pellet shapes. Nevertheless, there is potential for the development of even more flavorful compressed rice pellets in the future. Based on this research, there is a possibility to enhance the value of rice for farmers.

5. Acknowledgement

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