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Total phenolic content and phytochemicals of broken landrace rice and their utilization in food products

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Abstract

The aim of this research was to investigate the total phenolic content and phytochemical constituents of broken landrace rice flour from the Hom Dok Hang group, Ban Khok Sa-At, Sakon Nakhon province. Four types of rice—Red Jasmine, Black Jasmine, Kam Noi, and Hom Phu Keaw—were studied. The rice samples were crushed and sifted into two grain sizes: large grains, greater than 250 µm, and small grains, less than 250 µm. The rice samples were then extracted using ethanol as the solvent. The total phenolic content was determined, and the highest quantity of total phenolic content was found in the small grains of Red Jasmine flour, measured at 69.37 ± 2.22 mg GAE g^{-1} crude extract. For all rice types, the small grain size was found to have a higher phenolic content compared to the large grain size. The phytochemical was evaluated by qualitative analysis. It was found to contain anthraquinone, saponin, cardiac glycosides, terpenoids, flavonoids, coumarins, alkaloids, and tannins: however, steroids were not detected. Red Jasmine rice was selected as the main ingredient for product development. Kale powder and roasted black sesame powder were added to create a rice-based product targeted at menopausal women. The sensory properties of the product were evaluated, and the highest score was achieved with a recipe containing 85 g of Red Jasmine rice flour, 5 g of kale powder, and 15 g of black sesame powder. This study suggests that broken landrace rice flours are promising ingredients for food products, as they contain high levels of phytochemicals and antioxidants beneficial for health. Additionally, this approach adds value to raw materials that would otherwise be considered waste, such as broken rice, by transforming them into prototype food products.

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1. Introduction

Rice is the national food crop of Thailand, and an important export product essential to the Thai economy and rural communities. Thailand is known worldwide as one of the top producers and exporters of rice, and rice farming is a way of life in Thai culture [1]. Asia accounts for about 90% of global production. The major rice exporters are Thailand, India, and Vietnam [2]. Rice in Thailand can grow almost anywhere because of appropriate geography, which has caused genetic variety in rice throughout the country. Additionally, environmental factors in each area influence the growth of specific rice varieties and help establish their identity. In the Northeast, Sakon Nakhon Province has a group of community enterprises for the conservation and restoration of local rice varieties. The group name is Hom Dok Hang, which is a gathering of members of the Ban Khok Sa-At Farmers Group, Um Chan Subdistrict, Kusuman District. Their objective is to improve the quality of local rice seeds with the cooperation of the Sakon Nakhon Rice Research Center. Over 300 different types of rice have been generated by the use of natural compost in organic rice patches [3, 4]. In the rice production process, rice with full grains is sorted out for sale, which will get a good price. But the broken rice is not popular to eat or sell because the price is very low; so farmers will use it as animal feed. Sometime, the broken rice leaves go to waste; it may become moldy and cannot be used. The community group will process the broken rice into soap, shampoo, and grind it to make flour to sell.

The chemical composition of broken rice is slightly different from whole grain rice. Broken rice tends to have slightly higher protein, fat concentration, energy, and mineral content than whole polished rice because it removes the germ or pericarp layers [5]. The main component of broken rice is starch, at 87–91%, of which 6–25% is amylose and the remainder amylopectin [6]. The broken rice contains crude protein (7–12%), which is storage protein present in endosperm cells. In addition, 2–4% of fat was found in the form of essential unsaturated fatty acids [6, 7]. Broken rice is also a source of fiber, soluble carbohydrates, various minerals, and important phytochemicals such as flavonoids, phenolic acids, proanthocyanidins, ascorbic acid, and oxalic acids which are an antioxidant substance [6, 8]. Furthermore, broken rice is found to have abundant volatile components that have the highest odor activity value, such as alcohols, aldehydes, furans, ketones, sulfur, and terpenes [9].

Generally, broken rice leftover from milling is often used by farmers as animal feed, such as for pigs and piglets. For human food products, it is commonly processed into rice flour to make pasta, bakery products, cereals, and snacks that are gluten-free [10, 11]. Because broken rice lacks the gluten proteins that are present in wheat, barley, and rye, it's a suitable choice for people with celiac disease or gluten sensitivities [12]. In addition, broken rice is used in the beauty and cosmetic industries for skin cream, and in the textile industries for stiffening cloth [11].

Rice has also been developed into instant rice beverages for patients, the elderly, and perimenopausal women. The premenopausal age in the female population is between the ages of 45–55 years old, a stage classified as working age and approaching elderly status. The bodies of these women have dramatically changed, as their once-active ovaries cause estrogen levels to drop. Adverse side effects such as hot flashes, excessive sweating, irritability, and insomnia have been observed, including osteoporosis, high blood pressure, diabetes, and heart disease. Women of this age may preserve their balance by eating appropriate foods, such as foods containing phytoestrogens that can replace some of the lost estrogen of premenopausal people; foods containing antioxidants, less sugar, and intake plant-based protein; and food rich in important vitamins and minerals such as calcium, magnesium, and vitamin D [12, 13]. Phenolic compounds and phytochemicals have important roles in relieving menopausal symptoms and improving health during the menopausal period, such as promoting bone health by enhancing bone formation and inhibiting bone resorption, helping prevent menopausal osteoporosis [13, 14].

The objective of this study was to create added value for waste products such as broken landrace rice flour from the Hom Dok Hang group, Ban Khok Sa-AT, by studying the phytochemical constituents and total phenolic content. Then, broken landrace rice flour with antioxidants was used as raw material for rice products, replacing commercial flour. This research developed a healthy instant powdered drink product composed of broken rice flour, kale powder, and roasted black sesame powder as a source of calcium. Kale powder is a product from a community enterprise in Tao Ngoi district, Sakon Nakhon province. This powdered drink is a value-added product that is low-cost with a high potential for utilization.

2. Materials and Methods

Rice materials

The samples in this study were taken from the Hom Dok Hang group, Ban Khok Sa-At, Um Chan Subdistrict, Kusuman District, Sakon Nakhon Province. Four landrace rice varieties, including two types of broken rice, namely Red Jasmine and Black Jasmine rice, and two types of glutinous rice, namely Kam Noi and Hom Phu Keaw rice, were studied. The rice was crushed and sifted to separate it into two different flour grain sizes: large grain greater than 250 µm and small grains less than 250 µm (Fig. 1). Then, the rice flour was examined using a Scanning Electron Microscope (SEM) (Hitachi Model TM4000Plus) to observe the grain size and characteristics at 120x magnification with an accelerating voltage of 10 kV.

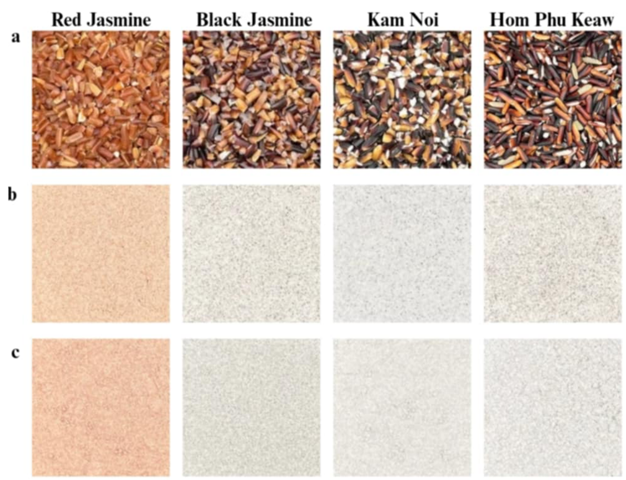

Fig. 1 Four landrace rice samples.
a. Rice grain, b. Large grain flour, c. Small grain flour

Sample extraction

The broken landrace rice flours were dried at 60 °C for 3 hours in a hot air oven. After drying, samples of approximately 50 g were extracted using absolute ethanol at a ratio of 1:3 (w v^{-1}) by the ultrasonic technique for 1 hour at 40 °C. Then, the samples were filtrated and dried to remove the solvent by rotary evaporator. The crude extract was kept in a reagent bottle at -20 °C for the next experiment. All of the broken landrace rice flour samples were processed in triplicate to ensure accuracy [15].

Total phenolic content

The total phenolic content of the samples was measured using the Folin-Ciocalteu colorimetric method [16]. Five mg of crude extract was diluted with 10 mL of absolute ethanol. After that, 200 µL of the extract was oxidized with 1 mL of 0.5 N Folin-Ciocalteu reagent and the reaction neutralized with 1 mL with 7.50% (w v^{-1}) Na_2CO_3. The sample mixture was incubated at room temperature for 2 hours and the absorbance measured at 760 nm with UV-Visible spectrophotometer (Genesys 180). The experiment was performed in triplicate. The total phenolic content in the crude extract samples was reported as milligrams of gallic acid equivalent (mg GAE) per g of crude extract, based on the standard curve of gallic acid. The total phenolic content among all treatments was analyzed using one-way ANOVA. Mean separation was performed using Tukey's HSD test at $p < 0.05$.

Phytochemical analysis

The broken landrace rice flour ethanolic extracts were tested for phytochemical constituents using standard procedures [17, 18].

Alkaloids: Add 0.5 g of ethanolic crude extract into a test tube; then add a few drops of Dragendorff reagent. A pink or red precipitate indicated a positive test.

Steroids: 0.05 g of ethanolic crude extract and 3 mL chloroform were added and filtered. 10 drops of ethanolic anhydride and 21 drops of concentrated H_2SO_4 were then added. A bluish green interphase indicated a positive test.

Terpenoids: 0.05 g of ethanolic crude extract and 3 mL chloroform were added and filtered. Ten drops of ethanolic anhydride and 21 drops of concentrated H_2SO_4 were then added. A pink interphase indicated a positive test.

Saponins: 1 mL Na_2CO_3 was added to 0.5 g of ethanolic crude extract; then a fehlings solution was added and warmed. The presence of brown precipitate indicated a positive test.

Cardiac glycosides: The Salkowski test was examined by dissolving 0.5 g of ethanolic crude extract with chloroform and adding concentrated H_2SO_4 to form a lower layer. A reddish-brown color at the interface indicated a positive test.

Tannins: 0.5 g of ethanolic crude extract was dissolved in distilled water, and ferric chloride reagent was added. A blue-black, green, or blue-green precipitate indicated a positive test.

Flavonoids: An ammonia test was performed by adding 0.2 g of ethanolic crude extract and 5 mL of ethyl acetate and then heating. After cooling and filtrating, 4 mL of the mixture was shaken with 1 mL of dilute ammonia solution. A color change indicated a positive test.

Coumarin: An NaOH test was performed by mixing 0.5 g of ethanolic crude extract with a 10% NaOH. A yellow color indicated a positive test.

Anthraquinone: 0.5 g of ethanolic crude extract was added to 1 mL of 10% H_2SO_4 and put in a water bath for 5 minutes. After cooling, 10% NH_3 was added. A pinkish red color indicated a positive test.

Development of rice-based products for menopausal women

The rice product was produced by roasting the broken rice at 70 °C for 3 minutes. It was then ground and sieved to a particle size of less than 250 µm [19, 20]. The rice flour, kale powder, and roasted black sesame powder were weighed according to the ratios shown in Table 1.

Table 1 The mixture of rice flour, kale powder, and roasted black sesame powder (g).

Recipe	Rice flour	Kale powder	Black sesame powder
A	92.5	5	2.5
B	90	5	5
C	87.5	5	7.5
D	85	5	10

It's been reported that one teaspoon of kale powder, weighing approximately 5 g, is equivalent to about 100 g of fresh kale. This amount is recommended for people who are new to consuming kale powder. The suggested daily intake is 5–10 g day^{-1} [21]. Therefore, this study used 5 g of kale powder per experiment. As for black sesame, the recommended daily limit is 15 g because it has high energy content. In this experiment, the maximum amount used was 10 g.

A sensory evaluation was conducted covering five aspects: appearance, texture, aroma, taste, and overall acceptance. The assessment involved 25 inexperienced sensory panelists, who were 45–55 years old and rated their preference levels using a 9-point hedonic scale. The sample with the highest sensory score was analyzed for chemical composition, including moisture, fat, protein, ash, fiber, total carbohydrates, and energy, according to AOAC [22] methods. Additionally, a microbiological analysis was performed to determine mold, yeast, and coliform bacteria counts in triplicate samples, following the Bacteriological Analytical Manual Online [23].

3. Results and Discussion

Characteristics of broken rice flours

After rice samples were finely ground, the rice flour was examined under SEM to observe the characteristics. It was found that the large grain sample was shaped like irregular polygons, while the small grain sample contained irregular particles (Fig.2). The polygonal shape represents granules tightly packed within the rice endosperm [24, 25].

A rice grain consists of an inedible husk on the outside. The inner part, called the brown rice grain, contains a bran layer, a germ, and starch in the center [26]. During milling, the bran is removed first, but the starchy core is hard and more difficult to grind. Some reports have shown that a higher milling level indicates a greater percentage of bran removed, which contains oils, vitamins, minerals, antioxidants, and protein [16, 27]

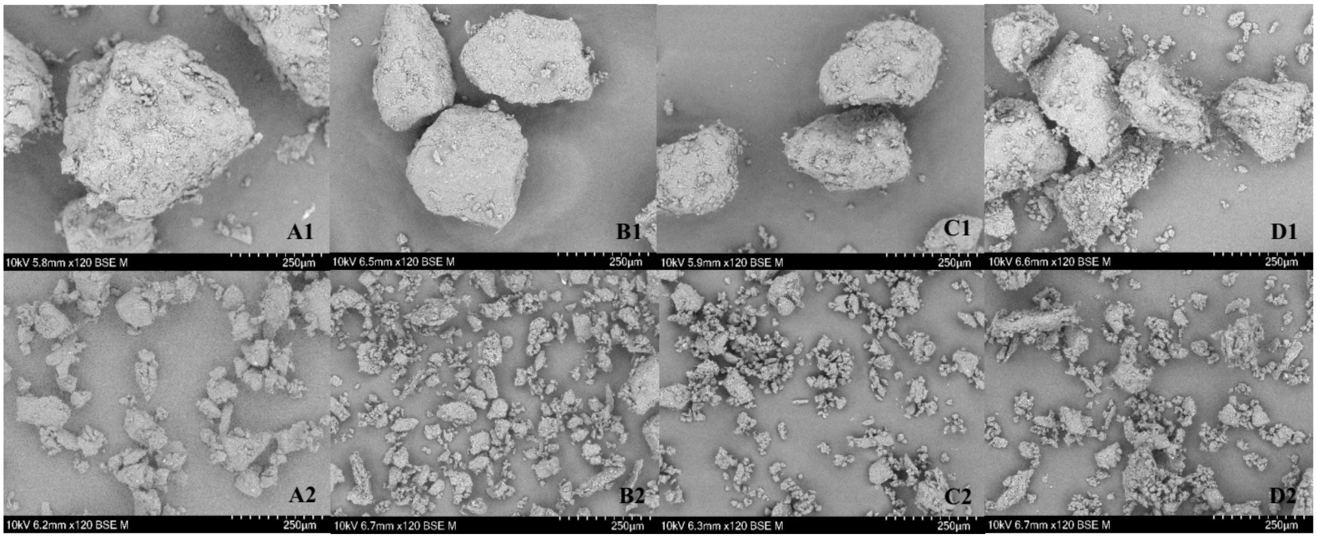

Fig. 2 Scanning electron microscope (SEM) images of rice samples: A1– A2 are large and small grain Red Jasmine; B1 – B2 large and small grain Black Jasmine; C1 – C2 large and small grain Kam Noi; D1 – D2 large and small grain Hom Phu Keaw.

The quantity of total phenolic content

A study of the total phenolic content extracted by using the absolute ethanol and ultrasonic technique found that the small grain rice flour had a higher phenolic content than the large grain flour. Smaller grains expose more surface area, facilitating better solvent penetration during extraction. They may contain a large amount of rice bran and rice germ, thus a higher recovery of phenolic compounds [16, 28]. This result was similar to the findings of Yafang *et al.* [16], who studied the total phenolic content and antioxidant capacity of small rice grains and found that smaller grains had higher phenolic content, flavonoid content, and antioxidant capacity than normal and larger grains.

Table 2 Total phenolic content of four landrace rice.

Sample		**Total phenolic content (mg GAE g^{-1} crude extract)**
Red Jasmine	Small grain	69.37 ± 2.22^{a}
	Large grain	14.51 ± 4.09^{bc}
Black Jasmine	Small grain	17.16 ± 2.83^{bc}
	Large grain	12.97 ± 0.36^{c}
Kam Noi	Small grain	16.65 ± 1.78^{bc}
	Large grain	11.84 ± 0.71^{c}
Hom Phu Keaw	Small grain	20.19 ± 2.77^{b}
	Large grain	11.98 ± 1.66^{c}

Note: The value of total phenolic content is presented as mean ± SD. Different letters indicate significant differences by Tukey's HSD test at $p < 0.05$.

The highest phenolic concentration in this study was observed in the small grain of Red Jasmine flour, at 69.37 ± 2.22 mg GAE g^{-1} crude extract, which was significantly different from other samples (Table 2). These results showed higher quantities when compared with the findings of Suwannatrai *et al.* [15], who studied the total phenolic content of roasted broken brown rice powder from Ban Khok Sa-At. They reported a result of 20.40 mg GAE g^{-1} crude extract. In addition, a study by Tikapunya *et al.* [29] showed the total phenolic content of Red Jasmine rice from Lamphun Province in a range of 30.58–31.76 mg GAE g^{-1} of extract. The reason that Red Jasmine rice has a high phenolic content may be due to environmental factors. A report by Shoa *et al.* [30], which studied the interaction between phytochemicals and antioxidants with genotype and environmental factors in 14 red rice varieties, found that total phenolic content is affected by environmental factors. Weather and biotic stress, such as infections and pests, impact the antioxidant levels that are important to the plant defense system.

Phytochemical screening test

In the preliminary phytochemical evaluation by qualitative analysis, the broken landrace rice flour was found to contain anthraquinones, saponins, cardiac glycosides, terpenoids, flavonoids, coumarins, alkaloids, and tannins, while steroids were not detected (Table 3). This is similar to a report by Nawaz *et al.* [31], which referred to all types of rice as good sources of phytochemical compounds such as tocopherols, phytosterols, phenolic acids, flavonoids, etc. Similarly, Palis *et al.* [32] found that brown rice contained alkaloids, flavonoids, steroids, and anthraquinones. In addition, Ghasemzadeh *et al.* [33] observed phytochemicals among different pigmented rice brans and found phenolic compounds, coumaric acid, and flavonoids, reporting that the phytochemical content in black rice bran was higher than in red and brown rice bran. According to many studies, colored rice contains more phytochemicals and exhibits greater antioxidant activity than white rice [32–35]. In this research, the rice used in the experiment was colored rice, so various phytochemicals were detected. However, these compounds differed depending on the rice variety, which may have been due to genetic and environmental factors in the cultivation area [35].

Table 3 Phytochemical screening test of four landrace rice

Test	Red Jasmine		Black Jasmine		Kam Noi		Hom Phu Keaw	
	Small	Large	Small	Large	Small	Large	Small	Large
Alkaloids	-	-	-	-	-	-	-	+
Steroids	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+
Saponins	+	+	-	-	+	-	-	-
Cardiac glycosides	+	+	+	+	+	+	+	+
Tannins	+	+	-	+	-	-	-	-
Flavonoids	+	+	+	+	-	-	-	-
Coumarin	+	-	-	+	-	+	+	-
Anthraquinone	+	+	-	-	-	-	-	-

Note: - = Absent or negligible, + = Detected

Rice-based products for menopausal women

Based on the findings of these examinations, Red Jasmine was selected to develop a rice product for menopausal women. The ingredients of the product were Red Jasmine rice flour, kale powder, and roasted black sesame powder, formulated in four different recipes. After that, the sensory properties of the product were evaluated. It was found that Recipe D which contained 85 g of Red Jasmine rice flour, 5 g of kale powder, and 15 g of black sesame (Table 4) received the highest sensory score. The nutritional values of Recipe D were analyzed, revealing that crude carbohydrates were 73.81%, crude protein was 11.57%, moisture was 10.23%, crude fat was 2.47%, ash was 1.20%, and fiber was 0.72% (Table 5). The energy value was 364 kcal 100 g^{-1}. Additionally, the total microorganism count was found to be 10 colonies g^{-1} of sample, which does not exceed the Thai Community Product Standard for instant rice that specifies the total microorganisms count must not exceed 1×10^4 colonies g^{-1} of sample [36].

Table 4 Sensory evaluation of instant powdered drink from Red Jasmine rice.

Sensory evaluation	Recipe			
	A	B	C	D
Appearance	5.4 ± 1.07	5.7 ± 0.95	5.9 ± 1.19	6.5 ± 1.65
Texture	6.0 ± 0.67	5.6 ± 0.97	5.7 ± 1.06	6.0 ± 1.49
Aroma	6.1 ± 1.45	6.0 ± 0.94	6.0 ± 1.05	6.4 ± 1.50
Taste	6.3 ± 1.25	6.0 ± 0.94	6.3 ± 1.33	6.4 ± 1.65
Overall acceptance	6.1 ± 0.99	6.0 ± 0.67	5.9 ± 0.87	6.4 ± 1.50

Note: The value is presented as mean ± SD.

Table 5 Nutritional value of instant powdered drink from Red Jasmine rice.

Nutrients	Value
Crude carbohydrate	73.81 %
Crude protein	11.57 %
Moisture	10.23 %
Crude fat	2.47 %
Ash	1.20 %
Fiber	0.72 %

This powdered rice drink is an energy drink that uses carbohydrates as its main component and can be consumed as a light meal. The Department of Health, Ministry of Public Health, has defined dietary principles for menopausal women, recommending the consumption of rice or unpolished rice to increase dietary fiber intake and aid in the absorption of fats and bile in the intestines. Additionally, it is advised that women at this stage consume plant-based foods rich in phytoestrogens because they have estrogen-like structures that help balance hormones and function similarly to estrogen [37]. Since rice itself contains only small amounts of these compounds, kale was incorporated into the product as an additional ingredient. Furthermore, ground black sesame was added as a source of calcium to support bone and dental health [38].

4. Conclusion

The broken landrace rice flours, Red Jasmine, Black Jasmine, Kam Noi, and Hom Phu Keaw, were found to contain phenolic content and various types of phytochemicals that contribute to antioxidant capacity. The small grains exhibited higher total phenolic content than the large grains. The Red Jasmine small grain demonstrated the highest total phenolic content and was selected to develop an instant powdered drink for premenopausal women. The primary ingredient, broken Red Jasmine rice flour, serves as a source of carbohydrates, dietary fiber, antioxidants, and protein. Additional ingredients, including kale and black sesame powders, contribute other valuable nutrients such as calcium and phytoestrogens. Thus, broken landrace rice flours show potential for use in food products similar to commercial flours. This approach is considered innovative, as these flours contain various phytochemicals and high concentrations of antioxidants beneficial to health. In particular, Red Jasmine rice can be applied in the cosmetic industry, especially anti-aging products; health food industry; and other industries. Moreover, this research adds value to local agricultural rice products that are low cost or treated as waste by utilizing them as raw materials.

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6. Declaration of generative AI in scientific writing

This manuscript used generative artificial intelligence (AI) tools, namely QuillBot for grammar checks. All scientific content, analysis, and conclusion were developed by authors.

7. CRediT author statement

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8. Research involving human and animal rights

This study was conducted in accordance with ethical principles for research involving humans and animals, following the guidelines of relevant institutional regulations to ensure the protection, safety, and welfare of participants and animals.

9. Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee for Research Involving Human Subjects No. HE 068/009 on March 12, 2025. Written consent was obtained from all participants before the start of the study. Participants were assured that their information would be kept confidential.

10. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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