# Physical Characteristics, Phytochemical Contents and Antioxidant Activity of Ten Organic-Pigmented Rice Varieties from Surin Province

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### Abstract

The objective of this study was to investigate the physical characteristics, phytochemical contents, and antioxidant activity of 10 organic-pigmented rice varieties including Tubtim Chumpae, Niaw Daeng, Nieng Guang, Mali Daeng, Hom Nil, Niaw Dam, Riceberry, Mali Gomain Surin, Malinil Surin, and Pa Ga Am Puen (obtained from Tapthai organic villages, Tha-mo, Prasat, Surin province, Thailand in June, 2021). The physical characteristics investigated included grain length, width, shape, and color in both paddy and unpolished rice. The results revealed that the mean length and width of paddy rice grain varieties ranged from 6.58 to 10.85 mm and 2.12 to 3.27 mm, respectively, while the length and width of unpolished rice grain were 5.94 to 7.53 mm and 1.74 to 2.61 mm, respectively. Most organic-pigmented rice varieties had a slender shape except for Nieng Guang and Niaw Dam, which were moderately shaped. There were 5 colors of paddy rice, including straw, purple, purple straw, black and black furrow on straw background and light purple straw while unpolished rice showed red, dark purple, black, purple, and light brown color. The anthocyanin content, total phenolic compounds and antioxidant activity of different rice varieties showed a significant difference in the range of 4.38 to 77.96 mg/100 g DW, 42.94 to 341.19 mg GAE/100 g DW and 2.71 to 34.77 mM TE/100 g DW, respectively. Niaw Dam exhibited the highest anthocyanin concentrations. The highest total phenolic content was found in Mali Daeng and Nieng Guang, resulting in high antioxidant activity. This study suggested that pigmented rice is rich in phytochemicals and antioxidants. Nieng Guang, in particular, could be promoted for cultivation and beneficial use in the food, pharmaceutical, and cosmetic industries.

Keywords: Physical characteristics, Phytochemicals, Antioxidant activity, Organic brown rice, Pigment rice, Indigenous rice, Commercial rice

# Introduction

Rice (Oryza sativa L.) is a staple food, economic crop, and widely cultivated in all parts of Thailand. Thung Kula Ronghai, in particular, covers 5 northeastern provinces in Thailand, namely Roi Et, Yasothon, Maha Sarakham, Sisaket, and Surin [1] and is recognized as the most well-known area for the highest quality production of Khao Hom Dawk Mali 105 (KDML 105, a commercial cultivar of aromatic rice. Surin province is one of the major KDML 105 producing areas classified as a geographical indication (GI). In addition, there is a diversity of pigmented rice varieties available, both indigenous and commercial. Niaw Daeng, a glutinous rice variety with plant height of 165 cm and a total yield of 400 - 500 kg/rai, is one of the indigenous rice varieties known and still cultivated in Surin province. This variety is planted in the Thung Kula Ronghai area in June. Nieng Guang is a non-glutinous rice which the Khmer people who live in the lower northeast of Thailand cultivate. The Khmer word of Nieng Guang means "not to be lost or persistent", considering the auspicious rice is used as the main ingredient in various merit-making ceremonies. This rice variety has a strong medium stem with purple color, green leaves with purple veins and a plant height of 120 - 140 cm. The Ga Am Puen rice variety whose name means "tamarind blossom", is a non-glutinous rice that comes from the Thai-Cambodian border and is commonly grown in Surin province of Thailand and Udon MiChai province of Cambodia. The characteristics of the variety are tolerance to dryness, the paddy is of light purple straw color, a slender grain, and is soft and fluffy when cooked. Pigmented commercial rice is rice that has been bred to match consumers' preferences in terms of physical characteristics, cooking, texture properties, high nutritional value, pest-resistance and high yield. Pigmented commercial rice popularly cultivated consists of the non-glutinous rice variety Tubtim Chumpae or RD69, which is obtained by crossbreeding 2 varieties of KDML 105 and Sangyod Phatthalung. This is a non-photoperiod variety with a plant height of 113 cm, a sturdy upright and variegated stem, green leaves as well as high yield, good cooking and eating qualities. Mali Daeng is a non-glutinous rice obtained from a mutant of KDML 105. It is a photoperiod sensitive variety with an erect and very sturdy stem, a plant height of 120 - 130 cm and light green leaves. Furthermore, it is soft, and sticky and has an odor similar to KDML 105. The Hom Nil rice variety has been bred by researchers at Kasetsart University. This is a nonglutinous rice variety with a short harvest period of about 95 days after seeding, a plant height of about 50 cm, dark green and purple leaves and stems, is soft and sticky when cooked, and has a good fragrance. Niaw Dam is classified as a glutinous rice with black colored husk. It has short harvesting time that can be done around mid-October. The stem is very sturdy and erects with a plant height about 151 cm and green leaves. Riceberry is classified as a non-glutinous rice obtained from crossbreeding Hom nil with KDML 105. This variety can be planted all year round, has harvesting maturity of about 130 days, and is resistant to blast disease. When cooked, it has an aromatic flavor and soft texture. Mali Gomain Surin is a nonglutinous pigmented rice obtained from the selection of native rice Mali Daeng no. 54 variety in the field at Surin Rice Research Center. It is a non-photoperiod sensitive variety with a plant height of about 153 cm, a sticky and soft texture as well as a good fragrance when cooked. Malinil Surin is obtained from the selection of native pigmented fragrant rice (Mali Dam no. 53) in the field at Surin Rice Research Center. This variety has an erect stem with a plant height of about 120 cm and light green leaves [2].

Surin province's pigmented rice production is much smaller than that of KDML 105 because Thai and Southeast Asian consumers prefer rice with good aroma, long grain, and soft texture, whereas pigmented rice is typically hard and without fragrance when cooked (although it has great health benefits) [3]. In general, Thai consumers like to cook pigmented rice by mixing it with KDML 105. Therefore, improving pigmented rice will provide high cooking quality for consumers' satisfaction and acceptance. It is possible to increase the amount of consumer demand as well as its value of being processed into confectionery and rice noodles (Khanom Jeen) food supplements and cosmetics [4,5]. Nowadays, consumers are focused more on health-conscious products. Thus, it has become more common to consume pigmented organic rice such as black, purple, brown, and red due to these varieties having a high content of bioactive compounds [6] such as anthocyanin, total phenolic, and flavonoid [7]. In addition, several studies have reported that pigmented rice contains higher levels of carotenoid [8,9] Vitamin E [10] and essential minerals including copper, calcium, iron, manganese, zinc, potassium and magnesium than the white rice [11-13]. These secondary compounds mentioned above have been shown to possess antioxidant properties which reduce cardiovascular diseases, cancer, and have antiviral, anti-inflammatory and free radical scavenging activity [14,15]. Das and Oudhia [16] found that an Indian indigenous pigmented rice variety "Laicha" protected skin from eponymous skin disease and "Kavuni" variety showed anti-arthritic, anti-diatetic and anti-oxidative properties, and was effective in curing gastritis and peptic ulcers as well as inducing blood circulation in the body [17]. However, the difference in the amounts of phytochemicals in pigmented rice grain is affected by both genetic and environmental factors. The color of rice grain is regulated by different family and structural genes. Sweeney et al. [18] found that Rc gene relates to the encoding of bHLH transcription factor and Rd gene to coding of dihydroflavonol 4-reductase which is the enzyme involved in the synthesis of proanthocyanidin resulting in the red color of rice grain. According to the report of Furukawa et al. [19], the Rc-Rd genotype is present in the red grain and the Rc-rd genotype is observed in the brown grain. Meanwhile, the black and purple rice grains are a result of anthocyanin which is controlled by 2 groups of bHLH family genes such as Ra1, OsB1, Rb, Ra2, and OsBs, as well as the R2R3-MYB gene (OsC1) [20]. Thitipramote et al. [21] demonstrated that red rice (Brown Red Jasmine) had higher total phenolic content and antioxidant activity than black rice (Kam Leum Pua) with proanthocyanidin found in red rice while black rice showed anthocyanidin. In addition, many studies have found that environmental factors such as biotic and abiotic stress, production system, light, temperature, water, and soil characteristics control the bioactive compound biosynthesis in rice grain. Sangwongchai et al. [22] reported production systems and soil characteristics effect on the grain quality and starch properties of KDML 105 and RD 6. In addition, water activity also affects the accumulation of phytochemicals and micronutrients in rice. Jaksomsak et al. [23] showed that purple rice grown under a wetland condition had higher anthocyanin, Zn, and Fe than in aerobic conditions due to flooded soil inducing biosynthesis of anthocyanin. These results were similar to those reported by Xu et al. [24] who found that the nonpigmented rice grown under well- watered region had a higher concentration of Zn, Fe, Mn, Mo, Se and Cu in bran and milled rice than the rice grown in moderately dried region. Previous studies have reported that nutritional accumulation in rice is regulated by an interaction between genetic and environmental factors. Shao et al. [25] studied the effect of genotype and environment on phytochemicals and antioxidant capacities of fourteen red rice varieties cultivated in Hangzhou (summer season) and Hainan (winter season) province, China. The results showed that the genotype and environment interaction affected TPC, TFC,

TPAC, ABTS, and DPPH radical scavenging capacities in all red varieties. In Indonesia, the Toraja local black rice from West Java had the highest content of total anthocyanins, phenols, quinones, flavonoids, anthraquinones, leucoanthocyanidins, and glucosides compared to other rice varieties from Java Island [26]. Settapramote *et al.* [27] reported that Riceberry rice from Lampang province, Thailand had a higher content of anthocyanin and antioxidant activities than the rice from Chiang Mai, Phetchabun, Sing Buri, and Surin provinces. Besides, the nutritional value of pigmented rice is a significant factor in consumer decision-making. The visual and physical characteristics of rice grain, such as color, shape, and size are among the factors that affect the purchasing decision of the consumer and the determination of rice standards for export. The physical properties are still being used in the classification of rice grains because it is clear and quick to check [28]. The purpose of this study was to analyze ten varieties of commercial and indigenous pigmented rice still cultivated at Tapthai organic village, Tha-mo, Prasat, Surin province, Thailand and determine their physical characteristics and nutritional value. The basic information obtained might be useful for selecting rice varieties for future rice breeding. The pigmented rice nutrition information, especially indigenous pigmented rice, has potential application in the food, pharmaceutical, and cosmetic industries.

#### Materials and methods

#### **Rice samples and chemicals**

Ten organic-pigmented rice varieties, namely Tubtim Chumpae, Niaw Daeng, Nieng Guang, Mali Daeng, Hom Nil, Niaw Dam, Riceberry, Mali Gomain Surin, Malinil Surin, and Pa Ga Am Puen were obtained from Tapthai organic village, Tha-mo, Prasat, Surin province, Thailand in June 2021 (**Figure 1**). All samples were grown during the wet season at Tapthai organic village, Tha-mo, Prasat, Surin province in 2020. The rice cultivation and postharvest handling were done under the organic rice production system with recommendations as follows: 1) Cultivating green manure plants such as legumes, cowpeas and sward to improved soil property, 2) Using biological, including animal manure and compost to increase soil nutrients, 3) Using physical, biological or combination method to control pest, and 4) Using clean and hygienic equipment and tools for harvesting, milling and processing.

Hydrochloric acid, potassium chloride, sodium acetate, ethanol, methanol, and sodium carbonate were purchased from Merck (Germany). 2,2-diphenyl-1-picrylhydrazyl,2,4-dinitrophenol, gallic acid, folicciocalteu's reagent, and Trolox were purchased from Sigma-Aldrich (USA). The chemicals used in this study were of analytical grade.

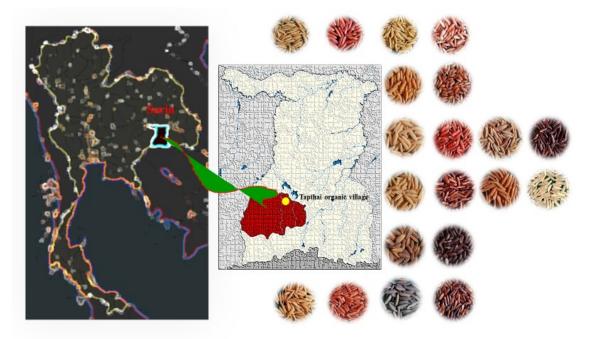


Figure 1 The map of Tapthai organic village, Tha-mo, Prasat, Surin Province, Thailand, where the pigmented organic rice was obtained [29].

Both the paddy and unpolished rice grain color tests were performed according to the method of Naivikul [30]. The color of paddy rice consisted of straw, yellow, brown straw, brown furrow straw background, brown, light purple, purple straw, black furrow on straw background, purple and black. The unpolished rice grain colors were: White, light brown, dark brown, red, purple, dark purple, and black.

The lengths and widths of 30 grains of both paddy and unpolished rice of each variety were measured (in mm, from the base to the tip of grains and the widest point of the grains, respectively) using a digital Vernier caliper.

The ratio of grain length/width was used to describe the grain shape as follows: slender shape (L/W > 3.0), medium shape (L/W 2.1 - 3.0), short (L/W 1.1 - 2.0), and round shape (L/W < 1.0) [31].

#### Phytochemicals content and antioxidant activity

Anthocyanin content was measured according to the method of Wrolstad *et al.* [32]. The 1 g each of pigmented rice flour was extracted with 10 mL of 0.1 % H<sub>2</sub>SO<sub>4</sub> in methanol and then sonicated at 40 °C for 30 min. Rice extracts (0.5 mL) were mixed with 2.5 mL of 0.025 M potassium chloride buffer with pH 1 and 2.5 mL of 0.4 M sodium chloride buffer with pH 4.5 and kept in the dark for 15 min at room temperature. The absorbance was scanned at  $\lambda_{vis-max}$ , and  $\lambda_{700}$ . The anthocyanin content was calculated using the following equation.

Anthocyanin (mg/100 g DW) =  $\frac{A \times MW \times DF \times 100}{\varepsilon x 1}$ 

where  $\epsilon = 26900$  molar extinction coefficient, in L.mol<sup>-1</sup>cm<sup>-1</sup>, for cyanidin-3-glucoside A=  $\Delta$  Abs MW = 449.2 g mol<sup>-1</sup> for cyanidin-3-glucoside DF = Dilution factor

Total phenolic content was determined according to a slightly modified method of Cheung *et al.* [33]. Pigmented rice flour (0.2 g) was mixed with 20 mL of 80 % ethanol and incubated at 50 °C for 1 h. The solutions were centrifuged at 2000 g for 20 min. For analysis, 300  $\mu$ L of rice extracts were diluted in 1.2 mL of distilled water followed by 6.25  $\mu$ L of 2N Folin-Ciocalteu's phenol reagent then 6.25  $\mu$ L of 7.5 % Na<sub>2</sub>CO<sub>3</sub> addition to the reaction mixture then incubation at room temperature for 90 min. The absorbance was measured at 725 nm using a 1601 UV-visible spectrophotometer. The results were expressed as gallic acid equivalents in mg GAE/100 g DW

Antioxidant activity (DPPH method) was done according to Pintatum *et al.* [34]. The samples (0.2 g) were mixed with 20 mL of 80 % ethanol and incubated at 50 °C for 1 h then centrifuged at 2000 g for 20 min. Thereafter, 0.05 mL of supernatant was mixed with 0.2 mL of 0.1 mM DPPH in ethanol and 2.25 mL of 80 % ethanol and incubated in the darkroom for 30 min. The absorbance was then measured at 517 nm. The standard curve was linear between 0 to 1.0  $\mu$ M Trolox.

#### Statistical analysis

All data obtained were analyzed statistically to find the physical property, phytochemicals and antioxidant activity of organic-pigmented rice using a Completely Randomized Design (CRD). There were 10 treatments with 4 replications. Each repeated used as 500 g sample. Statistical analysis was performed using SAS 9.0 (SAS Institute Inc. Cary, NC) for variance (ANOVA) and means were compared using Duncan's New Multiple Range Test (DMRT).

#### **Results and discussion**

#### **Physical characteristics**

The physical characteristics (including color, shape, length, width, and length/width ratio) of paddy and unpolished organic-pigmented rice are shown in **Tables 1** and **2**, and **Figures 2** and **3**, respectively. The color of ten paddy rice was dissimilar in the different varieties. The paddy of Tubtim Chumpae, Niaw Daeng, Mali Daeng, Hom Nil, and Mali Gomain Surin had straw colors. Nieng Guang was purple in color, Niaw Dam and Pa Ga Am Puen were purple straw, Riceberry was black and Malinil Surin was black furrow on straw background. Unpolished rice revealed that 4 varieties, Tubtim Chumpae, Niaw Daeng, Nieng Guang, and Mali Daeng had a red color among pigmented rice grains. Hom nil showed dark-purple color, Niaw Dam and Malinil Surin were black, Riceberry and Mali Gomain Surin were purple while Pa Ga Am Puen was a light brown color. The pigments are localized in the pericarp and bran of rice kernels resulting in different colors of rice such as black, purple, red, brown, or yellow which affect anthocyanins, proanthocyanidins, and carotenoids [35]. The color that appeared in all rice varieties depended on genetics. Several studies have found that the pigment of grain is regulated by many genes with the *Rc-Rd* genotype found in red rice grain, *Rc-rd* genotype found in brown rice grain and 2 groups of *bHLH* family genes (*Ra1, OsB1, Rb, Ra2 and OsBs*) and *R2R3-MYB* gene (*OsC1*) found in black and purple rice grains [18, 19]. The anthocyanins, proanthocyanidins, and carotenoids present in pigmented rice are compounds known to have high nutritional value. According to the reports of Pramai and Jiamyangyuen [36], pigmented rice had higher antioxidant capacities and bioactive compounds than that of non-pigmented rice because of its antioxidant contents.

The mean length of paddy rice ranged from 6.58 to 10.85 mm with the longest grain found in Niaw Daeng and the shortest grain was Nieng Guang, while the length of unpolished rice ranged from 5.94 to 7.53 mm. Pa Ga Am Puen had the longest grain (7.53 mm) followed by Niaw Daeng (7.44 mm) and Mali Daeng (7.33 mm) while the shortest grain was found in the Nieng Guang variety (5.94 mm). This study revealed that most paddy rice had longer grain length than unpolished rice because of the pointed tip of the paddy rice compared to the round tip of unpolished rice.

Paddy rice widths ranged from 2.12 to 3.27 mm, with Niaw Dam having the widest at 3.27 mm, while unpolished rice widths were the widest in Niaw Dam (2.61mm). Tubtim Chumpae and Riceberry showed the lowest grain widths at 1.96 and 1.98 mm, respectively.

The ratio of grain length/width was used to classify the grain shape of paddy and unpolished rice as a whole grain. The grain shape of most samples (8 varieties) was slender (L/W > 3.0) except for those of Nieng Guang and Niaw Dam varieties, which were moderately shaped (L/W = 2.1 - 3.0). While rice breeding was primarily aimed to increase crop productivity, Rao et al. [37] argued that the grain shape is one of the physical properties that are focused on in rice breeding to meet consumer's demand. In addition, the size, shape and amylose content of grain were considered to improve grain quality. However, the physical characteristics of grains differed between rice varieties depending on their genetic characteristics. Additionally, environmental factors e.g., soil properties, production systems, and climatic conditions [38,39] as well as postharvest handling, may affect the physical qualities of rice. Yuliana and Akhbar [40] reported that white and pigmented rice cultivation in different areas of West Java, West Sumatra, and Tangerang provinces in Indonesia exhibited variation in physical and physicochemical properties. The physical properties of rice indicate the rice grain quality and its market value. In addition, rice grain quality demonstrates the importance and potential for improving rice to obtain new quality rice varieties in terms of physical appearance, cooking and texture properties as well as nutritional value, etc., in order to achieve the highest consumer acceptance [41]. A previous study by Custodio et al. [3] reported that Thai consumers and Southeast Asian consumers prefer rice that has a good aroma, long-grain, and soft texture. Moreover, the physical properties of rice are essential during handling, storage, and processing of rice [42].

#### **Phytochemical contents**

Anthocyanin synthesis in rice grains involves 2 groups of structural genes and regulatory genes. The structural genes synthesize enzymes, including anthocyanidin synthase (ANS), dihydroflavonol 4-reductase (DFR), chalcone synthase (CHS), chalcone isomerase (CHI), flavonoid 3-hydroxylase (F3H), flavonoid 3glucosyl transferase (3GT) and phenylalanine ammonia-lyase (PAL). A previous study by Saika et al. [43] found that the *DER* gene is a late step of the anthocyanin synthesis pathway which is associated with the conversion of phenylalanine to anthocyanin. In addition, 2 groups of regulatory genes such as bHLH family genes (Ra1, OsB1, Rb, Ra2, and OsBs) and the R2R3-MYB gene (OsC1), control the expression of the structural genes in anthocyanin biosynthesis. Black and purple rice grains are attributed to the anthocyanin pigment [18,19] with the color intensity of grain differing depending on the gene regulating anthocyanin production [44]. The Rc-Rd genotype showed in red rice grain, and the Rc-rd genotype was found in the brown rice grain [19]. Genetic variability affects the diversity of pigmentations, nutritional value, and phytochemical properties in rice grain [45]. Several studies have reported that black rice has the highest anthocyanin content due to the pigment found in its bran and the outer layer being anthocyanin, while proanthocyanins are found in red rice and brown rice, and carotenoids are found in light brown, yellow, and white rice [46-48]. Ghasemzadeh et al. [49] reported that black rice bran showed the highest levels of total anthocyanin contents followed by red and brown rice. That black pigmented rice such as Khao Kam, Hom Kanya, Homnil, Khao Dam Leum Pue, Khao Kam Poon, and Red Hom Mali contains the highest amount of anthocyanin [50,51]. Pereira-Caro et al. [46] also studied the phytochemical properties of black, red, brown and white rice from the Camargue area, south of Arles (France). They found that black rice had a high content of anthocyanin, red rice had a high level of procyanidins whereas brown and white

rice showed the lowest amount of phytochemicals. Total anthocyanin content of glutinous rice such as Niaw Dam Pleuk Dam, Niaw Dam Pleuk, Kum Doi Saket, and Leum Pua ranged from 119 to 442 mg/100 g which was higher than in non-glutinous rice, including Hom Nil, Riceberry and Malinil Surin which ranged from 65 to 129 mg/100 g [36, 52-56]. The present study revealed that anthocyanin content ranged from 4.38 to 77.96 mg/100 g DW. Niaw Dam showed the highest anthocyanin concentration (77.96 mg/100 g DW) followed by Malinil Surin (37.53 mg/100 g DW). The lowest levels of anthocyanin were found in Pa Ga Am Puen (4.38 mg/100 g DW) whereas no difference was found in 5 varieties of Tubtim Chumpae. Niaw Daeng, Mali Daeng, Nieng Guang, and Mali Gomain Surin (Figure 4). This result was similar to many previous studies that have conclusively confirmed that each variety of rice has different genetic characteristics. The amount of anthocyanin was significantly different in black rice, which had the highest anthocyanin content. Moreover, some environmental factors also affect anthocyanin content such as planting area, production system, light, temperature, water and soil properties. Fongfon et al. [57] reported that N and Zn fertilizer management was appropriate for purple rice as a guideline to increase yield, N, Zn, and anthocyanin content in grain. A study of Kushwaha [58] revealed that anthocyanin content in black rice increased with an increase in the altitude of the cultivated area. The anthocyanin content in Riceberry rice from various locations in Thailand, including Chiang Mai, Lampang, Phetchabun, Sing Buri, and Surin province, was higher than in rice grain from Lampang province which is about 500 m above sea level [27]. Thus, these results suggested that anthocyanin is abundant in purple and black rice, where grain pigment is more intense than in red and light-brown rice. Genetic and environmental interactions affect the anthocyanin content of rice grain. Furthermore, the management of organic rice production systems, such as soil management, organic fertilizer, and so on, affects the accumulation of anthocyanin content in grain because organic fertilizers provide N or other plant nutrients at a lower rate than chemical fertilizers, resulting in purple and black organic rice with a low anthocyanin content in comparison to the chemical system.

Variety	Type of rice	Rice husks color	Shape	Length (L) (mm)	Width (W) (mm)	L/W ratio
Tubtim Chumpae	NG	straw	slender	10.30 <sup>bc</sup>	2.32 <sup>de</sup>	4.43°
Niaw Daeng	G	straw	slender	10.85 <sup>a</sup>	2.54 <sup>bcd</sup>	4.27 <sup>e</sup>
Nieng Guang	NG	purple	medium	$6.58^{\mathrm{f}}$	2.73 <sup>b</sup>	2.41 <sup>j</sup>
Mali Daeng	NG	straw	slender	10.18°	2.55 <sup>bcd</sup>	3.99 <sup>g</sup>
Hom Nil	NG	straw	slender	10.35 <sup>bc</sup>	2.60 <sup>bc</sup>	3.98 <sup>h</sup>
Niaw Dam	G	purple straw	medium	8.16 <sup>e</sup>	3.27ª	2.49 <sup>i</sup>
Riceberry	NG	black	slender	10.55 <sup>ab</sup>	2.39 <sup>cd</sup>	4.97 <sup>b</sup>
Mali Gomain Surin	NG	straw	slender	10.59 <sup>ab</sup>	2.12 <sup>e</sup>	4.99ª
Malinil Surin	NG	black furrow on straw background	slender	9.41 <sup>d</sup>	2.13°	4.41 <sup>d</sup>
Pa Ga Am Puen	NG	light purple straw	slender	8.88 <sup>de</sup>	2.09 <sup>g</sup>	4.24 <sup>f</sup>
F-test				**	**	**
C.V. (%)				1.78	7.60	10.59

Table 1 Physical characteristics of ten organic-pigmented paddy rice varieties from Surin province.

Values in the same column followed by a different letter are significantly different (p < 0.01)



**Figure 2** Shape and color of (a) Tubtim Chumpae, (b) Niaw Daeng, (c) Nieng Guang, (d) Mali Daeng, (e) Hom Nil, (f) Niaw Dam, (g) Riceberry, (h) Mali Gomain Surin, (i) Malinil Surin, and (j) Pa Ga Am Puen organic-pigmented paddy rice.

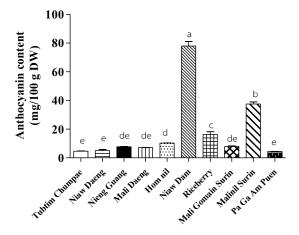
Variety	Unpolished rice grain color	Shape	Length (L) (mm)	Width (W) (mm)	L/W ratio
Tubtim Chumpae	red	slender	7.28°	1.96 <sup>e</sup>	3.71 <sup>b</sup>
Niaw Daeng	red	slender	7.44 <sup>ab</sup>	2.14 <sup>b</sup>	3.47°
Nieng Guang	red	medium	5.94 <sup>g</sup>	2.14 <sup>b</sup>	2.77 <sup>h</sup>
Mali Daeng	red	slender	7.33 <sup>bc</sup>	2.10 <sup>bc</sup>	3.49°
Hom Nil	dark purple	slender	7.28°	2.10 <sup>bc</sup>	$3.46^{\mathrm{f}}$
Niaw Dam	black	medium	$6.23^{\mathrm{f}}$	2.61ª	2.38 <sup>i</sup>
Riceberry	purple	slender	6.91 <sup>e</sup>	1.98 <sup>de</sup>	3.48 <sup>d</sup>
Mali Gomain Surin	purple	slender	7.14 <sup>d</sup>	2.05 <sup>cd</sup>	3.48 <sup>d</sup>
Malinil Surin	black	slender	7.28°	1.74 <sup>e</sup>	4.18 <sup>a</sup>
Pa Ga Am Puen	light brown	slender	7.53 <sup>a</sup>	2.03 <sup>bc</sup>	3.70 <sup>c</sup>
F-test			**	**	**
C.V. (%)			1.03	2.15	3.12

Table 2 Physical characteristics of ten organic-pigmented unpolished rice varieties from Surin province.

Values in the same column followed by a different letter are significantly different (p < 0.01)



**Figure 3** Shape and color of (a) Tubtim Chumpae, (b) Niaw Daeng, (c) Nieng Guang, (d) Mali Daeng, (e) Hom Nil, (f) Niaw Dam, (g) Riceberry, (h) Mali Gomain Surin, (i) Malinil Surin, and (j) Pa Ga Am Puen organic-pigmented unpolished rice.

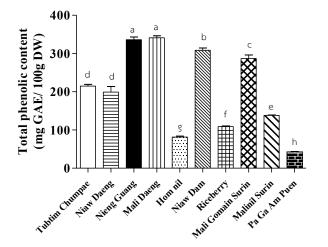


Tubtim Chumpae	4.59 mg/100 g DW
Niaw Daeng	5.05 mg/100 g DW
Nieng Guang	7.72 mg/100 g DW
Mali Daeng	7.09 mg/100 g DW
Hom nil	10.27 mg/100 g DW
Niaw Dam	77.96 mg/100 g DW
Riceberry	16.36 mg/100 g DW
Mali Gomain Surin	7.84 mg/100 g DW
Malinil Surin	37.53 mg/100 g DW
Pa Ga Am Puen	4.38 mg/100 g DW

Figure 4 Anthocyanin content in ten varieties of organic-pigmented rice. Vertical bars represent SD of means. Different letters indicate significant differences (p < 0.01).

Phenolic compounds, also known as polyphenols, are secondary substances found in plants. Their structure consists of 1 or more aromatic rings connected by 1 or more hydroxyl groups [59]. There are 2 forms of phenolic compounds found in rice grains, soluble and insoluble [60]. Generally, the phenolic compounds have been isolated and analyzed in black, purple, and red rice using HPLC or UPLC-ESI-QqQ-MS/MS chromatogram whereby vanillic acid,  $\rho$ -coumaric acid, protocatechuic acid, caffeic acid, ferulic acid, catechin and quercetin were found [11,61-63]. These compounds are beneficial to human health and play an important role in antioxidant activity and reducing the risk of cancer, diabetes, cardiovascular and neurodegenerative diseases [64-66]. Many previous studies have found that black and red rice have higher

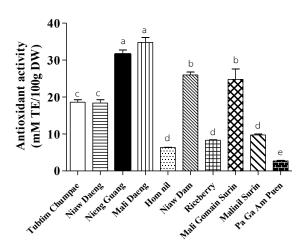
levels of total phenols than white rice, and black rice has higher levels of phenolic acid than red rice [65,67,68]. Pengkumsri et al. [69] studied bioactive compounds and antioxidant properties of Chiang Mai black rice, Suphanburi-1 brown rice, and Mali red rice from the northern part of Thailand. It was found that Chiang Mai black rice had higher phenolic content (detected by the HPLC) than other rice varieties with the compounds being protocatechuic acid, caffeic acid, syringic acid, and p-coumaric acid. Mali red rice only had p-hydroxybenzoic acid, while Suphanburi-1 brown rice had protocatechuic acid, chlorogenic acid, and syringic acid. The total phenolic content in Kum Doi Saket (KDK) purple rice was 0.2 times higher than CMU 125) purple rice. Meanwhile, Red Hawm rice (RHR) and Khao Dawk Mali 105 (KDML 105) white rice had total phenolic content 0.2 - 0.6 times lower than KDK and CMU125 rice [70]. Similar findings of the total phenolic content were found in black rice, Taichung-176, Khunmal-4, and Jumli Marsi red rice in Nepal. The black rice had the highest total phenolic content (22.75  $\pm$  0.02 g GAE/100 g DW) followed by Jumli Marsi red rice, Khunmal-4, and Taichung-176 which were  $15.47 \pm 0.03$ ,  $11.74 \pm 0.01$ and  $9.35 \pm 0.01$  g GAE/100g DW respectively [71]. The native pigmented rice in Thailand also showed that Khao Dam, and Leum Pua rice had higher levels of total phenolic content than other black rice varieties [50]. In contrast, the results showed that Mali Daeng had the highest level of total phenolic content at 341.19 mg GAE/100 g DW with no significant difference from Nieng Guang (336.28 mg GAE/100 g DW). Pa Ga Am Puen had the lowest total phenolic content (42.94 mg GAE/ 100 g DW) (Figure 5). Mali Daeng and Nieng Guang red rice varieties had the highest content of total phenols compared with the other samples. Tubtim Chumpae and Niaw Daeng, both red rice, had lower total phenolic content due to phenolic compound fragmentation in rice varieties, which each variety having its own phenolic compound profile. For example, phenolic acid in red rice determined using reversed-phase HPLC revealed that the red rice contained protocatechuic acid but no vanillic acid [63]. Previous studies of Waewkum and Singthong [50] showed Red Hom Mali (red) had higher phenolic content compared to Homnil (black) and Hom Mali (brown). Thitipramote et al. [21] reported that Brown Red Jasmine (BRJ) red rice contained the highest amount of total phenolic, which was 10 times more than Kam Leum Pua (KLP) black rice and 14 times higher than Japanese brown rice (JBR). In addition, Shao et al. [25] reported that environmental factors affecting total phenolic content in red rice accounted for 75 % of the total variance, while genetic traits accounted for 23 % with both abiotic and biotic stress also as factors. Variations in phenolic content were found in cereal crops, including rice, barley, oat, and sorghum, growing in different geographic locations [72]. Postharvest handling may also impact the total phenolic content of the rice. Mardiah et al. [73] explained that a 1-time polishing process or half-aleurone removal improved the eating quality of red rice. On the other hand, this process reduced the total phenolic content in red rice by up to 54 %. Similar 2 dehusked and medium polished Kamdhari, Black basumati, Sirsi and Jyothi red rice varieties in India showed a high content of polyphenolics [74]. These results indicated that total phenolic content in organicpigmented rice from Surin may vary with its phenolic acid profile, varieties, the grain colors as well as geographic locations or plantation sites, abiotic and biotic stress, and rice polishing process.



Tubtim Chumpae Niaw Daeng	214.99 mg GAE/100 g DW 199.26 mg GAE/100 g DW
Nieng Guang	336.28 mg GAE/100 g DW
Mali Daeng	341.19 mg GAE/100 g DW
Hom nil	81.39 mg GAE/100 g DW
Niaw Dam	308.45 mg GAE/100 g DW
Riceberry	108.84 mg GAE/100 g DW
Mali Gomain Surin	284.81 mg GAE/100 g DW
Malinil Surin	138.15 mg GAE/100 g DW
Pa Ga Am Puen	42.94 mg GAE/100 g DW

Figure 5 Total phenolic content in ten varieties of organic-pigmented rice. Vertical bars represent SD of means. Different letters indicate significant differences (p < 0.01).

Antioxidant activity in each organic-pigmented rice grain was measured by DPPH assay. This is the easiest method to measure the ability of antioxidants to inhibit and eliminate free radicals. The principle of free radical elimination by DPPH method is based on the electron transfer from the donor molecule to the free radical [75]. The present study found that both Mali Daeng and Nieng Guang had higher antioxidant activity than the other varieties. Pa Ga Am Puen had the lowest antioxidant activity (Figure 6). Several studies have reported that pigmented rice is rich in phytochemicals, including flavonoid,  $\gamma$ -oryzanol, carotenoid, tocopherols, anthocyanins, and polyphenolic compounds, which play a role in antioxidants [21,69,71,76]. Ghasenzadeh et al. [49] explained that 7 black rice cultivars, such as RB211, RB218, RB222, RB225, RB233, RB246, and RB248, showed the highest DPPH activity, followed by red and brown rice, which also had the lowest  $IC_{50}$  value, which contained the highest level of flavonoid and polyphenol compounds. Black rice variety in Nepal had the highest total phenolic content and antioxidant activity [71]. Boonyanuphong and Tobgay [76] reported that the glutinous black rice showed higher total phenolic content, total flavonoid content, and anthocyanin content than the red rice, resulting in high antioxidant activity. On the other hand, the present study showed that the highest antioxidant activity was observed in the red rice genotype, namely Mali Daeng, and Nieng Guang. According to many previous studies, the antioxidant capacity in red rice was higher than in black and white rice [25]. Brown red jasmine (BRJ) rice showed higher DPPH activity than Kam Leum Pua (KLP) black rice and Japanese brown rice (JBR) [21]. Both Chiang Mai Black rice (CBLR) and Mali Red rice (MRR) had the highest antioxidant activity, while Suphanburi-1 Brown rice (SBrR) showed the lowest antioxidant activity [67]. Waewkum and Singthong [50] described that most red and black rice had higher antioxidants than non-pigmented rice, with Red Hom Mali (RHR) showing higher antioxidant activity than Riceberry (RBR), Homnil (HNR), and Hom Mali (HR) respectively. The antioxidant activity increased with an increase in the content of flavonoid and phenolic compounds. Huang and Lai [77] also explained that the RB-1<sup>st</sup> red rice showed higher antioxidant activity than black rice. Antioxidant activity had a high correlation with total phenolic content at r = 0.987of DPPH radical scavenging activity and total flavonoid content at r = 0.889 of DPPH radical scavenging activity. Many studies have indicated that rice varieties with high phenolic compounds are responsible for their antioxidant activity. Moreover, Chen et al. [78] explained in detail that benzene ring which contains the same substitute as the carboxylic group (RCOOH) had a direct effect on the antioxidant activity of phenolic acid. Therefore, the phenolic acids profile is divided into 3 groups according to their ability to donate electrons or radical scavenging. Hydroxyphenylacetic acid (-CH<sub>2</sub>COOH) had stronger antioxidant activity than hydroxycinnamic acid (-CH=CHCOOH), and hydroxybenzoic acid (-COOH). Protocatechuic acid, isovallic acid, vanillic acid, and syringic acid belong to the benzoic acid group, while p-comaric acid, caffeic acid, isoferulic acid, ferulic acid, and sinapic acid belong to the cinnamic acid group, and homoisovanillic acid, homoisovallic acid, and 3,4-dihydroxyphenylacetic acid belong to the phenylacetic. As a result, the antioxidant activity of pigmented rice changes in relation to the total phenolic content.



Tubtim Chumpae Niaw Daeng	18.60 mM TE/100 g DW 18.37 mM TE/100 g DW
Nieng Guang	31.56 mM TE/100 g DW
Mali Daeng	34.77 mM TE/100 g DW
Hom nil	6.33 mM TE/100 g DW
Niaw Dam	25.97 mM TE/100 g DW
Riceberry	8.32 mM TE/100 g DW
Mali Gomain Surin	24.78 mM TE/100 g DW
Malinil Surin	9.74 mM TE/100 g DW
Pa Ga Am Puen	2.71 mM TE/100 g DW

Figure 6 Antioxidant activity in ten varieties of organic-pigmented rice. Vertical bars represent SD of means. Different letters indicate significant differences (p < 0.01).

#### Conclusions

The findings of the present study revealed that the shape of ten varieties of organic-pigmented rice were classified as slender (Tubtim Chumpae, Niaw Daeng, Mali Daeng, Hom Nil, Riceberry, Mali Gomain Surin, Malinil Surin, and Pa Ga Am Puen) and moderate (Nieng Guang and Niaw Dam). There were 5 colors of paddy rice, including straw, purple, purple straw, black and black furrow on straw background and light purple straw while unpolished rice showed red, dark purple, black, purple, and light brown color. Niaw Dam black rice exhibited the highest anthocyanin concentrations (77.96 mg/100 g DW), while the highest levels of total phenolics were found in Mali Daeng and Nieng Guang red rice, resulting in high antioxidant activities. Pa Ga Am Puen, light brown rice, had the lowest phytochemicals and antioxidant activity. Future studies should focus on the utilization of pigmented rice in food, pharmaceutical, and cosmetic industries, particularly Nieng Guang, a native rice variety in Surin province, to add value and promote cultivation.

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