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Physicochemical, Functional and Antioxidant Properties of Pigmented Rice

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Abstract

Pigmented rice has hight antioxidant nature, and its properties contribute to the successful formulation of nutraceutical food products. Based on this background, this research was aimed at determining the physicochemical, functional and antioxidant properties of ten pigmented Indonesian rice varietiels - Merah Saleman, Mota, Beureum Taleus, Anak Doro M, Cempo Salamet, Ketan Putri, Ketan Hitam I, Ketan Hitam II, Gogo Niti II, and Care Hitam. The physic properties and proximate analysis of pigmented rice were showed a significant difference at $p<0.05$, except for crude fiber. The sample with the lowest amylose showed the highest peak, trough, and final viscosities, but with low pasting temperature. The gelatinization enthalpy ranged from 0.82 - 1.33 J/g, and the sample with the highest amylose had the lowest gelatinization peak. The functional properties of the water absorption index ranged from 4.22 - 7.63 g/g, the water solubility index was between 3.62 - 7.40%, the oil absorption index varied from 0.88 - 1.36 g/g, while the swelling power was between 5.31 - 8.42 g/g. Furthermore, the antioxidant properties of the samples measured in terms of the phenolic content ranged from 2.17 - 10.60 mg GAE/g, the flavonoids varied from 0.43 - 2.31 mg QE/g and their activity used the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals. The total flavonoid in the phenolic component was positively correlated with the antioxidant activity. In conclusion, the ten pigmented rice showed a wide range of physicochemical, functional, and antioxidant properties, making the samples useful for the formulations of various products with health benefits.

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Introduction

Public interest has increased regarding pigmented rice because it has been found to perform health-benefiting biological activities, including those who are anti-mutagenic, anti-lipid peroxidation, anti-inflammatory, aldose reductase inhibitor, cholesterol absorption inhibitor, anti-hepatic steatosis, and reactive oxygen species suppressor.[1] As such, pigmented rice is a popular crop for nutraceutical products because it contains high levels of antioxidant compounds, including phenolics, anthocyanin, and gamma-oryzanols.

Antioxidants from pigmented rice can neutralize free radicals, transfer anti-aging benefits, and prevent diseases related to oxidative stress.[2] Recent research states that pigmented rice has been applied as an indigenous medicine.[3] Because of these health benefits, there has been increasing interest in developing pigmented rice-based food products for human consumption.

According to Falade and Christopher[4], the types of rice flour used in various food products such as pudding, flatbread, beverages, ingredients in meat processing, salad dressing, and low gluten bread. In Indonesia, rice flour is used traditional foods like nagasari, talam, putu ayu, apem, and cacarbikang. Additionally, rice flour extracts are used as a food coloring in cake, alcoholic drinks, and a particular type of ice cream and puddings.[5] The development of novel foods products usually involves rice flours that have useful physicochemical and antioxidant properties that improve the functional foods products.[6]

Variations in the physicochemical, functional, and antioxidant properties of rice flour can influence the quality and contributes to the successful formulation of various food products. Physicochemical properties are known to change the cooking pattern as well as resulting food product texture.[7] Amylose has significant effects on gelatinization temperature, pasting behavior, and viscosity properties.[8] Functional properties such as the oil absorption index can be used shelf-life indicators because they are associated with rancidity, and the water absorption index indicates the texture of the food.[9] Antioxidant properties increase the functional value of the product because of their ability to neutralize free radicals.[10]

There are many cultivars of pigmented rice in Indonesia, and each has different properties. This research aimed to analyze the physicochemical, functional, and antioxidant properties of ten Indonesia pigmented rice. The results of the current study can be used as information to increase the value and quality of food products made from pigmented rice. From the point of view of plant breeding, diversity in physicochemical and antioxidant properties in pigmented rice is useful for global plant breeders to produce new lines with some desired specific traits.

Materials and Methods

Sample Collection

The ten local varieties of Indonesia pigmented rice (Merah Saleman, Mota, Beureum Taleus, Anak Doro Magelang, Cempo Salamet, Ketan Putri, Ketan Hitam I, Ketan Hitam II, Gogo Niti II, and Care Hitam) were cultivated under average climate conditions in the Agrotechnopark Research Center, Jember University, East Java, Indonesia and harvested in August 2018. All samples were finally collected in paper bags and were stored at −4°C.

Chemical Reagents

The chemical reagents used were selenium quercetin ($C_5H_{10}O_7$), gallic acid ($C_7H_6O_5$), folin-ciocalteu ($H_3PO_4(Mo_3)_{12}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid ($C_6H_8O_6$), 2-deoxy-d-ribose ($C_5H_{10}O_4$), hydrogen peroxide (H_2O_2), tertiary butyl alcohol (TBA), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA) procured from Sigma-Aldrich, Singapore.

Preparation of Samples

Rice grains were dehusked using rice huller and then polished using the rice mill machine (MB-RC52W, Yamamoto, Japan). The polished rice grains grounded to powder and then passed through a 60 sieve mesh (250 µm). All samples were finally placed in plastic bags and were stored at −4°C before analysis.

Physical Properties

1,000 Grains Weight (GW) and Length-Width Ratio (L:W)

The weight of 1,000 rice grains measured randomly using an analytical balance (EP-225 SM-DR, Precisa, Swiss). The vernier caliper was used the measure the length (mm) and width (mm). Length/width (L:W) ratio was calculated to determine grain shape. According to the IRRI (International Rice Research Institute),[11] rice grains classified into four categories: slender (>3 mm), medium (2.1-3.0 mm), bold (1.1-2.0 mm), and round (<1.0 mm).

Color Parameters

The color parameter was determined using a color reader (CR-20, Konica Minolta, US). The color reader was calibrated using a white calibration tile. Rice flour sample (35 g) placed in a clean petri dish and calculated. The parameters color were *L** (brightness to white), *a** (red to green), and *b** (yellow to blue).

Chemical Properties

Proximate Composition

The proximate composition of rice flour was estimated according to the AOAC method.[12] Protein content was determined by micro-Kjeldahl method, lipid content was determined by soxhlet apparatus method, ash content was determined by burning sample at 700°C in the electric furnace (PrepASH 229, Precesia, Japan), crude fiber content was determined AOAC method 978.10, and carbohydrate content was calculated based on the formula reported by Amagloh *et al.*,[13] Carbohydrate (%) = 100% - [Moisture (%) + protein (%) + lipid (%) + ash (%)]. Whereas, the gross energy was determined using the formula reported by Bhat and Yahya[14] Gross energy (kJ/100 g) = [Protein (%) + Carbohydrate (%) x 16,7 + Lipid (%) x 37.7].

Amylose Content

Amylose content determined by following the method of Juliano.[15] Rice flour (0.1 g) added by ethanol (96%, 1 mL) and NaOH (1 N, 9 mL). The solution was heated in the water bath (95°C 10 minutes) and then cooled (50 minutes). Solution (5 mL) taken and transferred to Erlenmeyer glass (100 mL). Acetic acid (1N, 1 mL), iodine (2%, 2 mL) and distilled water (92 mL) were added to the sample solution and then incubated (20-25°C, 20 minutes). The solution measured using UV-Spectrophotometer (U-2900, Hitachi, Japan) at 620 nm. The amylose was determined by comparing to a standard curve from potato amylose (mg/L).

Pasting Properties

Pasting properties were calculated using by Rapid Visco Analyzer (RVA-Techmaster, Perten, Australia) according to Kraithong method.[16] Rice flour sample (3 g) was placed in an aluminum canister. Distilled water (25 mL) was added before the solution was placed inside RVA. The programs of temperature in the following order: maintained (50°C, 1 minute) and then raised (95°C, 3.7 minutes). The sample was maintained (95°C, 2.5 minutes), cooled (50°C, 2.5 minutes), and maintained (50°C, 2 minutes). The plot of viscosity in RVA units (cP) versus time was used to determine peak viscosity (PV), trough viscosity (TR), breakdown viscosity (BD), final viscosity (FV), setback viscosity (SB), peak time (PTm), and pasting temperature (PT).

Thermal Properties

A differential scanning calorimetry (Rigaku, DSC8230, Japan) was used to determine the thermal properties of samples based on Siswoyo and Morita method.[17] Rice flour sample (2 mg) placed into DSC aluminum pan and then distilled water was added (Flour:water = 1:4). The sample was then hermetically sealed and allowed the stand (4°C, 1 hour). Software Thermo Plus EVO was connected and controlled the DSC machine, and then the sample was heated at a rate of 10°C/minutes in a nitrogen atmosphere (20 mL/minutes) from 35-120°C. Thermal transitions were defined to determine onset (To), peak (Tp), conclusion temperature (Tc), and gelatinization enthalpy (ΔHg).

Functional Properties

Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC) and Water Solubility Index (WSI) and

The WAC, OAC, and WSI were determined according to the methods established by Bhat and Yahya.[14] Sample of rice flour (2.5 g) were mixed with distilled water (20 mL) or corn oil (25 mL) and centrifuged (3,000 rpm, 15 minutes). The WAC, OAC, and WSI were calculated based on the formula:

WAC or OAC (g/g) = (Weight of water or oil absorbed (g))/(Weight of the sample (g))

WSI (%) = (Weight of dried supernatant (g))/(Weight of the sample (g)) X 100%

Swelling Power (SP)

The swelling power of rice flour was determined by Ali *et al.,* Method.18 sample (1 g) was dissolved into distilled water (10 mL) and incubated (60°C, 30 minutes). The sample was then cooled (25°C, 30 minutes) and centrifuged (3,000 rpm, 30 minutes). Swelling power determined by dividing the weight of pellets by the weight of the sample (g/g).

Swelling Power (g/g) = (Weight of pellets (g))/(Weight of the sample (g))

Antioxidant Properties

Total Phenolic (TP) and Flavonoid (TF) Content

Methanol (80%, 6 mL) was added to the unpolished rice flour (0.2 g) and macerated on the stirrer (4°C, 1 day). The solution was centrifuged (10,000 rpm, 10 minutes), and then the supernatant was taken and stored. The method described by Taga *et al.*,[19] was used to determine the TP. Extraction sample (5 µL) was added with Na_2CO_3 (2%, 1 mL), Folin Ciocalteu (50%, 50 µL), and methanol (100%, 45 µL). The mixture was then incubated at 24°C for 30 minutes, followed by measuring the absorbance at 750 nm using UV-Spectrophotometer (U-2900, Hitachi, Japan). Gallic acid was used as the standard (mg GAE/g).

The TF was estimated by Itidel *et al*., method.[20] Methanol (100%, 40 µL), distilled water (400 µL) and $NaNO_2$ (5%, 30 µL) were added to the extraction sample (10 µL). The solution was incubated at (24°C for 5 minutes); then, $AlCl_3$ (10%, 30 µL) was added and re-incubated at 24°C for 5 minutes. After the incubation, NaOH (1 N, 200 µL) and distilled water (240 µL) was added. The solution measured using Spectrophotometer (U-2900, Hitachi, Japan) at 415 nm. Quercetin was used as the standard (mg QE/g).

Antioxidant Activity of 2,2-Diphenyl-1-1Picrylhydrazyl (DPPH)

DPPH scavenging activity was measured using the method reported by Palombini *et al.,*[21] Serial samples extracts (10, 20, 40, 60 and 80 µg GAE/mL) were mixed with DPPH (90 µM, 400 µL) and distilled water until a volume of 500 µL. The solution was incubated in the absence of the light (24°C, 5 minutes). The absorbance was recorded at 517 nm using Spectrophotometer (U-2900, Hitachi, Japan). The percent inhibition was measured through a comparison to the control. The effective concentration (IC50) is the value of antioxidants to inhibit 50% activity of DPPH.

Hydroxyl Radical Scavenging

The scavenging activity of hydroxyl free radicals was determined using the method established by Halliwell *et al*.,[22] To the serial sample extracts (30, 60, 90, 120 and 150 µL) were added 2-deoxy-D-ribose (2,8 mM, 20 µL), Ethylenediaminetetraacetic acid (1 mM, 100 µL), $FeCl_3$ (10 mM, 10 µL), H_2O_2 (1 mM, 10 µL) and ascorbic acid (1 mM, 100 µL) and phosphate buffer until a volume 1 mL. The solution was then incubated (37°C, 1 hour). After the incubation, TBA (1%, 500 µL) and TCA (2.8%, 500 µL) were added and re-incubated (80°C, 30 minutes). The absorbance was measured by the spectrophotometer (U-2900, Hitachi, Japan) at 532 nm. The effective concentration (IC_{50}) is the value of antioxidants to inhibit 50% activity of DPPH.

Statistical Analysis

All experimental results were done in triplicates to test their reproducibility. The data was analyzed using SPSS version 6.0, and the result was presented as mean ± standard deviation (SD). Statistical analysis was done using ANOVA, while Tukey's test was used for post-hoc analysis. A significant difference was determined at $p<0.05$.

Discussion

Physical Properties

The physical properties of Indonesian pigmented rice is presented in Table 1. The GW of samples ranged from 12.85-25.65 g. The highest of GW was Ketan Putri (25.65 g), followed by Ketan Hitam I (23.83 g) and Merah Saleman (21.22 g), while the lowest was observed in samples from Anak Doro Magelang (12.85 g). GW values influenced by the environmental and atmospheric factors at the moment of grain harvesting.[23] Temperature is the most explored environmental aspect in relation to rice production and grain quality. GW range of 20-30 g for all samples indicates a high level of production.[24] 1000 grain weight is a critical measure of seed quality, which is useful on sprouting, seed potential, seedling growth, and plant performance.[25]

Table 1: Physical properties of Indonesia pigmented rice

Variety	GW* (g)	Length (mm)	Width (mm)	L:W ratio
Merah Saleman	21.22 ± 0.50^{d}	4.92 ± 0.05^{ef}	1.75 ± 0.04^{abc}	2.81 ± 0.06^{cd}
Mota	17.58 ± 0.65^{c}	3.99 ± 0.04^{a}	1.82 ± 0.04^{bcd}	2.20 ± 0.06^{a}
Beureum Taleus	16.77 ± 1.05^{bc}	4.41 ± 0.04^{bc}	1.91 ± 0.08^{cd}	2.31 ± 0.08^{ab}
Anak Doro M	12.85 ± 0.37^{a}	4.37 ± 0.04^{bc}	1.62 ± 0.07^{ab}	2.71 ± 0.09^{cd}
Cempo Salamet	16.63 ± 0.44^{bc}	4.58 ± 0.05^{cd}	1.56 ± 0.02^{a}	2.94 ± 0.03^{d}
Ketan Putri	25.65 ± 0.63^{f}	4.51 ± 0.06^{c}	1.99 ± 0.03^{de}	2.26 ± 0.05^{a}
Ketan Hitam I	23.83 ± 0.44^{e}	4.73 ± 0.11^{de}	2,17 ± 0.04^{f}	2,18 ± 0.09^{a}
Ketan Hitam II	15.71 ± 0.82^{b}	5.09 ± 0.10^{fg}	1.82 ± 0.06^{bcd}	2.80 ± 0.04^{cd}
Care Hitam	17.34 ± 0.29^{bc}	4.26 ± 0.07^{b}	1.54 ± 0.19^{a}	2.78 ± 0.27^{cd}
Gogo Niti II	17.47 ± 0.24^{c}	5.20 ± 0.10^{g}	2.00 ± 0.11^{de}	2.61 ± 0.11^{bc}

Data shown as mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$), *GW; 1000 grain weight and L:W; Length divider width.

The length of grains ranged from 3.99-5.09 mm, while the width ranged from 1.54-2.00 mm. All samples may be categorized under the category of short because they were less than 5.5 mm that. The L:W ratio analyzed to determine the grain shape and ranged from 2.18-2.94, which described all samples that have medium shape. The size and shape of rice grains were important for assessing the level of marketing because it is used as the first selection criterion in the variety of improvement program.[26]

Table 2: Color parameters properties of Indonesia pigmented rice

Variety	L*	a*	b*
Merah Saleman	64.20 ± 0.30d	4.13 ± 0.06^{d}	13.60 ± 0.10^{e}
Mota	65.43 ± 0.45^{e}	3.63 ± 0.15^{bc}	12.77 ± 0.06^{d}
Beureum Taleus	64.53 ± 0.21^{d}	3.97 ± 0.12^{cd}	14.37 ± 0.06^{f}
Anak Doro M	66.60 ± 0.20^{f}	4.30 ± 0.17^{d}	12.83 ± 0.06^{d}
Cempo Salamet	67.33 ± 0.12^{f}	3.27 ± 0.21^{b}	15.60 ± 0.17^{g}
Ketan Putri	64.47 ± 0.06^{d}	3.70 ± 0.10^{c}	12.37 ± 0.06^{c}
Ketan Hitam I	59.40 ± 0.20^{c}	0.73 ± 0.12^{a}	9.33 ± 0.12^{b}
Ketan Hitam II	58.23 ± 0.25^{b}	0.77 ± 0.15^{a}	8.77 ± 0.12^{a}
Care Hitam	57.37 ± 0.25^{a}	0.87 ± 0.06^{a}	9.07 ± 0.12^{ab}
Gogo Niti II	57.07 ± 0.29^{a}	0.80 ± 0.19^{a}	8.83 ± 0.21^{a}

Data shown as mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$), L*: Brightness, a*: Redness and b*: Yellowness.

The color parameters varied significantly across samples (Table 2). The Brightness (*L**) of samples ranged from 57.07-66.60. Gogo Niti II had the lowest brightness (57.07) followed by Care Hitam (57.37), Ketam Hitam II (58.23), and Ketan Hitam I (59.04). The most brightness of samples is Anak Doro Magelang (66.60). The red-green color quality (*a**) of samples was measured to be positive with a range from 0.73-4.13 that indicates all samples had the redness color. The blue-yellow quality of samples (*b**) was measured to range from 8.83-14.37. This range indicates that the samples were more yellow than blue. Color parameters can be attributed to genetic makeup. According to Anggareni *et al.,*[10] variations of grain color are influenced by phenolic compounds such as anthocyanins, which produce blues and purples, proanthocyanidins, which produce reds, and carotenoids which produce yellows. The strong pigmentation of the rice flour reflects the phenolic and flavonoid content, both of which yield health benefits such as the prevention of chronic diseases such as type II diabetes, obesity, and ailments.[27] Moreover, Shao *et al.,*[28] confirmed that there are correlations between L*, *a*, b** colors, and antioxidant compounds of pigmented rice.

Table 3: Proximate composition of pigmented rice

Variety	Ash (%)	Lipid (%)	Crude fiber (%)	Protein (%)	Carbohydrate (%)
Merah Saleman	1.33 ± 0.54^{ab}	1.32 ± 0.02d	1.22 ± 0.13^{a}	8.90± 0.13^{bc}	76.95 ± 0.79^{bc}
Mota	1.25 ± 0.18^{ab}	0.75 ± 0.16^{a}	1.31 ± 0.52^{a}	6.80 ± 0.39^{a}	78.74 ± 0.32^{cd}
Beureum Taleus	1.53 ± 0.15^{ab}	1.37 ± 0.05^{d}	1.01 ± 0.06^{a}	9.19 ± 0.38c	77.31 ± 0.45^{bcd}
Anak Doro M	0.95 ± 0.10^{a}	1.13± 0.14^{bcd}	1.08 ± 0.23^{a}	7.09 ± 1.06^{a}	78.80 ± 0.80^{d}
Cempo Salamet	1.08 ± 0.37^{a}	1.23± 0.02^{cd}	0.87 ± 0.35^{a}	10.91± 0.66^{d}	74.48 ± 0.73^{a}
Ketan Putri	0.93 ± 0.10^{a}	0.76± 0.15^{a}	1.51 ± 0.14^{a}	7.47 ± 0.25^{ab}	78.70 ± 0.43^{cd}
Ketan Hitam I	1.72 ± 0.15^{ab}	0.85 ± 0.09^{ab}	1.42 ± 0.19^{a}	7.91 ± 0.05^{abc}	76.65 ± 0.34^{b}
Ketan Hitam II	2.02 ± 0.28^{b}	0.64 ± 0.11^{a}	1.59 ± 0.28^{a}	7.53 ± 0.35^{ab}	78.28 ± 0.46^{bcd}
Care Hitam	1.58 ± 0.48^{ab}	0.76 ± 0.18^{a}	1.06 ± 0.35^{a}	8.55 ± 0.22^{bc}	76.77 ± 0.92^{b}
Gogo Niti II	1.20 ± 0.23^{ab}	0.91 ± 0.07^{abc}	0.95 ± 0.16^{a}	6.97 ± 0.61^{a}	77.68 ± 0.68^{bcd}

Data shown as mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different (p > 0.05).

Chemical Properties

Proximate composition represented in Table 3. The results of ash content ranged from 0.95-2.02% and presented the amount of mineral content in rice flour. The lipid, crude fiber, protein and carbohydrate content ranged from 0.64-1.32%, 0.87-1.59%, 6.80-10.91%, and 74.48-78.80%, respectively. The fiber and ash contents of the rice flour were related to the amounts of bran in the flour.[29] These results support those found by Kraithong *et al.,*[16] who measured the ash content at 0.47-1.44 %, the lipid content at 0.50-3.50%, and carbohydrate at 77.06-85.58%. Interestingly, crude protein content was measured to be lower than in the present study (1.58-6.22%). According to Moralez-martínes *et al.,*[30] protein content correlated with the texture of rice flour, where high levels of protein decrease the adhesiveness of cooked rice. The Carbohydrate content of all samples was greater than 70%, indicating that rice flour is a good energy source. The total energy represented in Table 4 ranged from 14441.97-14961.99 kJ/100g.

Amylose contents of rice a critical characteristic that affects necessary cooking procedures, pasting viscosity, and food texture.[16] The amylose content in this study was significantly different and varied from 2.85-26.14%. Cempo Salamet was found, and it has the highest amylose content, while Ketan Hitam II and Ketan Hitam I had the lowest. Lawal *et al.,*[31] classified their samples into four categories, where two varieties (Ketan Hitam II and Ketan Hitam I) had very low amylose content (2-12%), two varieties (Care Hitam and Mota) had low amylose content (12-20%), five varieties (Merah Saleman, Ketan Putri, Gogo Niti II, Beureum Taleus, and Anak Doro Magelang) had intermediate amylose content (20-15%) and 1 variety high amylose content (>25%).

Table 4: Gross Energy and Amylose content of pigmented rice

Variety	Gross Energy kJ/100g	Amylose (%)
Merah Saleman	14834.59 ± 50.96[cd]	20.95 ± 0.31[e]
Mota	14567.93 ± 212.29[ab]	17.88 ± 0.56[d]
Beureum Taleus	14961.99 ± 92.33[e]	22.91 ± 0.63[f]
Anak Doro M	14769.64 ± 268.21[abc]	24.58 ± 0.47[g]
Cempo Salamet	14723.84 ± 176.21[abc]	26.14 ± 0.35[h]
Ketan Putri	14676.91 ± 121.68[ab]	21.08 ± 0.40[e]
Ketan Hitam I	14441.97 ± 74.01[a]	4.87 ± 0.58[b]
Ketan Hitam II	14571.55 ± 146.68[bc]	2.85 ± 0.56[a]
Care Hitam	14534.62 ± 154.98[ab]	15.54 ± 0.39[c]
Gogo Niti II	14479.62 ± 74.01[ab]	23.11 ± 0.78[fg]

Data shown as mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$).

Rice with high amylose content exhibited high expansion volume during cooking, smooth, and become hard on cooling and rice with amylose content showed low expansion volume during cooking, rough, and become soft on cooling.[7] Besides, Rice with high levels of amylose content is suitable for hard texture food products such as extruded products, noodles, and snacks because it can provide crispness and firmness to the food product, while rice with low levels of amylose content suitable for meat products, puddings, and soft cakes because it can provide dampness and softness to the food product.[32]

Pasting Properties

Pasting properties of all samples is represented in Table 5. The peak, trough, and final viscosity in order were 1743.00-3892.00 cP, 1294.00-3271.00 cP, and 3260.00-5481.00 cP, respectively. Peak time and pasting temperature were at 6.83-8.00 minutes and 84.25-90.20°C. Ketan Hitam I has the highest pasting temperature, peak, trough, and final viscosity, but has a low peak time which has inversely proportional to Cempo Salamet. A high peak viscosity reflects the ability of rice flour granules to chain water through hydrogen bonds because it has much amylopectin, which is effected the capability of the swelling collapsing granule, which determined the texture product.[27,33] Pigmented rice with a height peak viscosity is suitable for liquid and soft textured food products such as gravy, salad dressing, puddings, and soft cakes. Rice flour with a low peak viscosity is suitable for products with a rough texture and weak elasticity.[34] The trough viscosity determines the viscosity of flour at a constant temperature, and the highest trough represents the least viscosity required to prevent a breakdown during refrigeration.[9] The final viscosity reflects the capability of paste to form a jelly after refrigeration and cooking. High final viscosity represents high resistance to shear tension throughout stirring.[32] According to Hsu *et al.,*[35] the final viscosity represented the stability of cooked flour paste and the ability to form a gel after cooling.

The breakdown of pigmented rice flour ranged from 309.00-1077.00 cP. Breakdown viscosity reflects the sensitivity of flour to disintegration, resistance against heat, and shearing after cooking.[4] Anak Doro Magelang has the lowest value for breakdown viscosity, indicating that the network structure of the molecules is easy to destroy. The setback viscosity of all samples ranged from 511.00-2333.00 cP. Setback viscosity reflects the re-crystallization of gelatin during cooling, or the tendency of flour paste to retrogradation.[37] Ketan Hitam II has the highest value of setback viscosity. This indicates a greater tendency for retrogradation over other variety. High breakdown and low setback viscosity value indicate good cooking quality because the cooked flour will not retrograde or become stiff when cooled.[36] The breakdown viscosity reflected flour paste resistance against heat and shear. Pasting temperature is the minimum temperature needed to form gelatin. Across all samples, the pasting temperature ranged from 84.25-90.20°C. Ketan Hitam I measured the highest pasting temperature, indicating that more considerable energy is needed during cooking. The peak time of all samples ranged from 6.40-7.47 minutes, and it determined the time of flour to pasting. Ketan Hitam II has the lowest peak time indicate the flour is easy to paste.

The compositions of pigmented rice influenced pasting properties. Amylose content can restrain swelling and increase the setback value because of the three-dimensional structure from the re-merging of amylose during refrigeration.[38] The other components that can influence the pasting properties are lipid and protein. Amylose-lipid and amylose-protein formation can increase the setback and final viscosity, while these can reduce peak and breakdown viscosity.[39]

Rice flour with a high amylose content exhibited a low peak, trough, and breakdown viscosity.[27] The other components that influenced the pasting properties are lipids and protein. The formation of complex bonds between amylose and lipids or amylose and proteins can decrease the peak and breakdown viscosity, but increase the setback and final viscosity.[39]

Thermal Properties

The pigmented rice variegated in thermal characteristics (Table. 5). The gelatinization onset temperature ranged from 78.30-84.10°C, peak temperature ranged from 80.90-88.00oC, conclusion temperature ranged from 84.60-91.30°C, and enthalpy ranged 0.82-1.36 J/g. The data was similar in that found by Odenigbo *et al.,*[40] who reported a gelatinization onset temperature ranging from 63.42-78.34°C, peak temperature ranging from 67.66-81.27°C, conclusion temperature ranging from 74.02-90.18°C and enthalpy ranging from 0.62-2.51 J/g. sample with high amylose content tends to exhibit high onset (T_0), peak (T_p), and conclusion (T_c) gelatinization temperature. The gelatinization endotherm interpreted the move from limited to maximum swollen of amylopectin molecules in flour granule and several dissolutions of amylose molecule in flour polymers. Ketan Hitam I exhibited the highest onset, peak, and conclusion gelatinization temperatures, all positively correlated with pasting temperature. Ketan Putri exhibited the lowest enthalpy, which reflects the small molecular weight. The endotherm is related to starch gelatinization and denaturation of the protein.[27] In addition, the thermal properties of rice flour are related to the presence of crystallinity.[41]

Table 5: Pasting properties of pigmented rice

Variety	PV (cP)	TR (cP)	BD (cP)	FV (cP)	SB (cP)	PTm (Minutes)	PT (°C)
Merah Saleman	2764.00	2081.00	683.00	3862.00	1098.00	7.47	87.83
Mota	2917.00	2589.00	328.00	4793.00	1876.00	6.66	88.25
Beureum Taleus	2876.00	1863.00	1013.00	3387.00	511.00	6.76	86.45
Anak Doro M	1862.00	1553.00	309.00	3459.00	1597.00	7.13	87.00
Cempo Salamet	1743.00	1294.00	449.00	3260.00	1517.00	7.68	84.25
Ketan Putri	2119.00	1962.00	157.00	4452.00	2333.00	7.47	87.36
Ketan Hitam I	3892.00	3271.00	621.00	5481.00	1589.00	6.97	90.20
Ketan Hitam II	3743.00	2684.00	1059.00	5473.00	2230.00	6.83	90.00
Care Hitam	3961.00	3253.00	708.00	4631.00	670.00	7.12	88.15
Gogo Niti II	2769.00	1692.00	1077.00	4457.00	1688.00	6.40	89.25

PV: Peak viscosity, TR: Trough viscosity, BD: Breakdown. FV: Final viscosity, SB: Setback, PTm: Peak time, PT: Pasting temperature

Table 6: Thermal properties of pigmented rice

Variety	To(°C)	Tp (°C)	Tc (°C)	ΔH_g (J/g)
Merah Saleman	81.50	84.40	87.70	0.91
Mota	82.80	86.60	89.70	1.26
Beureum Taleus	80.00	82.90	86.70	0.94
Anak Doro M	79.60	83.90	87.20	0.82
Cempo Salamet	78.30	80.90	84.60	0.88
Ketan Putri	80.50	83.80	87.20	0.82
Ketan Hitam I	84.10	87.10	90.70	1.05
Ketan Hitam II	84.00	88.00	91.30	1.36
Care Hitam	83.20	86.00	89.20	1.24
Gogo Niti II	82.40	85.30	88.40	0.86

To: Onset temperature, Tp: Peak temperature, Tc: conclusion temperature and ΔHg: gelatinization enthalpy

The variations in the thermal properties of pigmented rice are affected by amylose, amylopectin, lipid, and protein contents. The large amylopectin branch was reflected in the difficulty of destroying the large crystalline areas which are required large energy.[42] The formation of intermolecular covalent disulfide crosslinks made the proteins less soluble and less prone to bind with other flour.[43] Moreover, amylose-lipid and amylose-protein formation form a more rigid structure and thus required a higher temperature for gelatinization. An assessment of thermal properties was important for the applications of flour-based food products because they affect processing, elasticity, and swelling product.[30]

Functional Properties

The functional properties in this study is depicted in Table. 7. The WAC ranged from 4.22-7.63 g/g. The data were consistent with Kraithong *et al.,*[16] who published a WAC value between 5.44-7.14 g/g. The WAI reflects the ability of rice flour to bind to water molecules in limited water conditions.[40] The main factors that influence WAC values are carbohydrate, protein, lipid, and amylose content. The high carbohydrate and protein content in rice flour reflect a molecule structure that has a molar or charged side chain, which makes hydrogen bonds stronger, and the lipid content is revealed the structure of the molecule because it has a hydrophobic part which interrupts hydration of flour granule.[16] The size of granules significantly influences. Flour with small particle size has a big surface area to which the water molecules may bind. WAC is an important consideration for baking applications. Beureum Taleus had the highest WAC, which indicates that it is composed of low structure polymers that make food products soft and smooth. In contrast, Ketan Hitam I had the lowest WAC, associated with compactness in the polymer structure. These results are in agreement with Aprianita *et al.,*[44] who explained the highest WAI value indicated the flour has a high number of the hydrophilic group within flour molecules, and it provides softness and smoothness in the food product. In addition, the formation of a complex bond of amylose with lipids or proteins can interfere with water-binding capability through decreasing polarity of the charged molecule.[4]

The WSI range found in this study (3.62-7.40%) agreed with the findings of Kraithong *et al.,*[16] who reported the WSI of Thai organic rice flour ranging between 2.97-7.05%. The WSI value describes the capability of the sample to disperse in aqueous solution. Ketan Hitam I had the highest WSI, indicating that the sample was highly adhesive and sticky. Cempo Salamet has the lowest value for WSI, indicating the high capability to prevent food structure throughout cooked and caused stiff and hard in the food product. Chung *et al.,*[45] found that low WSI values were associated with zone junction formation by amylose, which resulted in a rigid flour granule structure. Besides, the formation of amylose-lipid complexes or protein-amylose can reduce the insoluble portion of flour, causing a decrease in the WSI values.

The OAC value results (0.88-1.36 g/g) were similar to those of Sarangapani *et al.*,[46] who reported OAC values ranging from 0.88-1.39 g/g. Care Hitam had the highest OAC value, indicating a relatively greater ability to the rice flour to binding oil molecules. OAC values are strongly influenced by protein and lipid content. Proteins are composed of hydrophilic and hydrophobic molecules. The side chains of non-polar amino acids with the hydrocarbon chain of lipids can form hydrophobic interactions.[9] Increasing levels of lipid content in rice flour can increase the OAC because of the greater of hydrophobic molecules. The OAC is important in food production because it indicates flour to maintain oil, which acts to retaining flavor, rancid taste, and enhanced mouthfeel.

Table7: Functional properties of pigmented rice

Variety	WAC (g/g)	WSI (%)	OAC (g/g)	SP (g/g)
Merah Saleman	6.52 ± 0.17^{d}	6.17 ± 0.13^{c}	0.90 ± 0.02^{a}	6.91 ± 0.06^{d}
Mota	6.04 ± 0.07^{cd}	5.06 ± 0.36^{b}	1.21 ± 0.03^{bc}	7.61 ± 0.10^{e}
Beureum Taleus	7.23 ± 0.08^{e}	5.55 ± 0.09^{bc}	0.88 ± 0.08^{a}	5.31 ± 0.09^{a}
Anak Doro Magelang	6.51 ± 0.10^{d}	5.35 ± 0.20^{b}	1.23 ± 0.03^{bcd}	6.62 ± 0.11^{d}
Cempo Salamet	7.63 ± 0.05^{e}	3.62 ± 0.23^{a}	1.12 ± 0.04^{b}	6.14 ± 0.07^{c}
Ketan Putri	5.82 ± 0.05^{c}	5.38 ± 0.46^{b}	1.34 ± 0.02^{d}	7.35 ± 0.17^{e}
Ketan Hitam I	4.22 ± 0.11^{a}	7.40 ± 0.24^{d}	1.16 ± 0.07^{bc}	8.42 ± 0.10^{e}
Ketan Hitam II	6.11 ± 0.60^{cd}	6.98 ± 0.18^{d}	1.11 ± 0.05^{b}	8.17 ± 0.13^{e}
Care Hitam	$5.13 \pm 0.\ 07^{b}$	5.71 ± 0.17^{bc}	1.36 ± 0.06^{d}	7.47 ± 0.22^{e}
Gogo Niti II	6.55 ± 0.24^{d}	5.58 ± 0.31^{bc}	1.27 ± 0.03^{cd}	5.74 ± 0.17^{b}

Data shown as mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$). WAC: Water absorption capacity, WSI: Water solubility index, OAC: Oil absorption capacity, and SP: Swelling power.

The swelling power of pigmented rice flour ranged from 5.31-8.42 g/g. The swelling power of flour indicates the level of associative power in flour granule and the water absorption index from the starch-based flour throughout cooking. Variations in swelling power are influenced by the ratio amylose to amylopectin, pasting properties, and molecules of flour, which have negatively charged phosphate groups.[18] High amylopectin is associated with increased swelling power. Higher protein content in rice flour caused rice granules to be embedded in a rigid protein structure, which restricts the hydrocarbons from binding to water molecules.[9] According to Chui and Zhu,[47] the simple sugars, proteins, lipids, dietary fibers, and minerals played a significant role in the swelling properties of the flours.

Antioxidant Properties

Total phenolic and flavonoid ranged from 2.17-10.60 mg GAE/g and 0.43-2.84 mg QE/g (Table 8.). The result from the test about antioxidants properties are similar to those found by Shen *et al.*,[47] who reported that the total phenolic content in pigmented rice ranged from 1.65-12.44 mg GAE/g for black rice and total flavonoid ranged from 1.08-2.86 mg QE/g. The percentage flavonoid in phenolic of all samples ranged from 17.63-26.77%. Ketan Hitam II has the highest total phenolic and flavonoids content, indicating that Ketan Hitam II has the highest healthy benefit.

Phenolic compounds are secondary metabolites in plants that have the scavenging ability due to the presence of hydroxyl groups. Phenolic compounds can decrease the oxidative rate of organic materials by transferring hydrocarbon atoms to radical molecules.[5] Flavonoids are a group of polyphenolic compounds whose biosynthesis can trough shikimic and malonic acid pathways. Flavonoids include enzymes such as aldose reductase and xanthine oxidase, which are powerful phenolic hydroxyl groups and antioxidants.[48] The flavonoids have antioxidant activities which when consumed protect against various forms of carcinogenesis and prevent cell damage and inflammation.[49]

Table 8: Antioxidant properties of pigmented rice

Variety	TP (mg GAE/g)	TF (mg QE/g)	TF/TP (%)	IC50 (µg/mL)	
				DPPH scavenging	Hydroxyl scavenging
Merah Saleman	3.23 ± 0.05^{b}	0.59 ± 0.03b	18.27 ± 1.21^{a}	61.06 ± 0.92^{f}	132.32 ± 2.98^{f}
Mota	2.17 ± 0.04^{a}	0.43 ± 0.02^{a}	19.63 ± 1.26^{ab}	51.33 ± 0.79^{e}	116.08 ± 1.56^{de}
Beureum Taleus	5.26 ± 0.10^{d}	0.93 ± 0.02^{d}	17.63 ± 0.38^{a}	67.28 ± 0.89^{g}	132.48 ± 2.88^{f}
Anak Doro M	4.80 ± 0.07^{c}	1.03 ± 0.03^{e}	21.51 ± 0.65^{bc}	58.84 ± 2.02^{f}	121.66 ± 2.47^{e}
Cempo Salamet	2,98 ± 0.05^{b}	0.67 ± 0.04^{c}	22.49 ± 1.39^{cd}	46.78 ± 0.69^{d}	111.00 ± 2.74^{d}
Ketan Putri	5.41 ± 0.06^{d}	1.07 ± 0.01^{e}	19.85 ± 0.35^{ab}	53.34 ± 1.33^{e}	101.55 ± 4.69^{c}
Ketan Hitam I	7.44 ± 0.17^{e}	1.68 ± 0.01^{f}	22.62 ± 0.35^{cd}	36.68 ± 2.08^{b}	99.54 ± 3.89^{c}
Ketan Hitam II	10.60 ± 0.34^{g}	2.84 ± 0.02^{h}	26.77 ± 0.72^{e}	31.03 ± 1.26^{a}	72.74 ± 0.76^{ab}
Care Hitam	7.19 ± 0.06^{e}	1.72 ± 0.03^{f}	23.96 ± 0.38^{d}	41.01 ± 0.86^{c}	77.48 ± 2.31^{b}
Gogo Niti II	9.51 ± 0.17^{f}	2.32 ± 0.03^{g}	24.35 ± 0.15^{d}	32.48 ± 1.85^{a}	68.61 ± 2.66^{a}

Data shown as the mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$), TP: Total Phenolic, TF: Total flavonoid. TF/TP: Percentage flavonoid in phenolic and IC50: The effective concentration of antioxidants to inhibit 50% activity of DPPH and hydroxyl radical.

DPPH is a free radical and becomes stable when received electrons or hydrogen from the flavonoid compound. The discoloration from purple to yellow indicates the capability of the sample to reduce radically.[50,51] In this study, IC_{50} presented the concentration of extract to reduce radical 50%. The result ranged from 31.03-67.28 mg GAE/g. The highest antioxidant activity of DPPH observed in Gogo Niti II, followed by Gogo Niti II, Ketan Hitam I, and Care Hitam. Hydroxyl radical produced by Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^*$), then hydroxyl reacted with 2-deoxy-D-ribose to form malondialdehyde which caused pink color. The presence of phenolic compounds caused the competition between phenolic and 2—deoxy-D-ribose to reacted with hydroxyl radical.

The reaction of hydroxyl and phenolic can reduce malondialdehyde and decrease the intensity of the pink color.[51] The result IC50 of Hydroxyl scavenging ranged from 68.61-132.48 mg GAE/g. Gogo Niti has the highest antioxidant, while Beureum Taleus has the lowest antioxidant. The various antioxidant activity influenced by genotype, grain color, and phenolic compound.[10] The advantages of antioxidants were oxidative lipid damage and low-density lipoproteins, which inhibiting platelet aggregation and reducing coroner heart and cancer diseases. According to Nam et al.,50 plants the main sources of antioxidant; however, pigmented rice has also been found as a good source of antioxidant components. The extract of all samples is the potential resource of nature. According to Supriyadi *et al.*,[52] (2019), natural resources with IC_{50} values bellow 500 µg/mL have great potential as nutraceutical resources. Besides, foodstuff and food products with high antioxidant activity attracted many people because of its superiority in the aspect of health.

Conclusion

The physicochemical properties of pigmented rice were significantly different. Amylose, lipid, and protein content in rice affected the pasting, thermal, and functional properties. The higher value of peak, trough, and final viscosities observed in low amylose flours but has a low pasting temperature. Lower thermal properties such as T_0, T_P, and T_C found in Anak Doro M, and Cempo Salamet promoted a relatively high amylose content. The result of the antioxidant properties of all samples showed a potential activity for free radical scavenging, mainly observed in Ketan Hitam II and Gogo Niti. Further work on formulation food products using pigmented rice is highly recommended. All data from this research can be used to promote pigmented rice variety as ingredients in functional food products for the health-conscious consumer.

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Conflict of Interest

The author(s) declares no conflict of interest.

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